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BIOINFORMATIKA

Sinteznobiološke nevronske mreže

Ana HALUŽAN VASLE[1*], Miha MOŠKON[1]

[1] Univerza v Ljubljani, Fakulteta za računalništvo in informatiko, Ljubljana, Slovenija

* ana.haluzanvasle@fri.uni-lj.si

Uvod: Nevronska omrežja možganov so bila navdih za vzpostavitev umetnih nevronskih mrež (ANN, angl. *artificial neural networks*), ki jih danes izkoriščamo na številnih področjih. Sintezna biologija je navdih za nove biološke gradnike in vezja pogosto črpala iz računalništva. V zadnjem desetletju smo tako prešli od preprostega biološkega preklopnega stikala do nevronskih mrež (NN, angl. *neural network*) na osnovi bioloških molekul. Priprava ustreznih bioloških gradnikov kot samih sinteznobioloških NN (SYNBIONN, angl. *synthetic biological neural network*) temelji na teoretičnih in eksperimentalnih pristopih. Teoretični pristopi se posvečajo pripravi, optimizaciji in *in silico* analizi bioloških vezij, ki poskušajo posnemati digitalna vezja in predstavljajo potencialne gradnike NN kot so programabilni (bio)logični bloki [1]. Eksperimentalne izvedbe so do sedaj večinoma temeljile na sistemih *in vitro*, osnovanih z DNA, RNA ali proteini [2-6], v letu 2022 pa je raziskovalcem uspelo preprosto SYNBIONN pripraviti tudi *in vivo* [7]. Omenjene izvedbe temeljijo na prednaučeni NN, ki omejuje uporabnost teh vezij v zahtevnih in hitro spreminjajočih se okoljih.

Materiali in metode: Cilj raziskave je priprava teoretičnega modela SYNBIONN, ki bo sposobna sprotnega učenja *in vivo.* V prvem koraku smo pripravili temeljit pregled do sedaj predstavljenih teoretičnih in eksperimentalnih mrež SYNBIONN ter med slednjimi izbrali nekaj najobetavnejših predstavnikov za nadaljnje preučevanje. Lotili smo se postavitve njihovih računalniških modelov in možnosti preoblikovanja ter nadgradnje teh *in silico.* Analizirali smo prednosti in pomanjkljivosti posameznih uporabljenih platform ter izpostavili možne ovire na poti do priprave idealne SYNBIONN.

Rezultati in razprava: Analiza izbranih obstoječih eksperimentalno potrjenih mrež SYNBIONN je pokazala, da sta stopnjevanje arhitekture in implementacija učenja zelo zahtevni. Za pripravo večplastnih mrež SYNBIONN bo najverjetneje potrebno posegati po sistemu, kjer bo vsako posamezno plast sestavljala drugačna vrsta bioloških molekul. Potencialno uporabni gradniki in arhitekture zahtevajo vzpostavitev standardiziranih metrik, ki

bi olajšale načrtovanje *in silico* in vrednotenje odziva pripravljenih mrež. Metrike za ovrednotenje posamezne arhitekture SYNBIONN bi zajemale oceno zmogljivosti, minimalno število potrebnih ortogonalnih delcev, število navzkrižnih reakcij, velikost končnega omrežja, ocene vhodnega in izhodnega območja delovanja, časovno zahtevnost in hitrost konvergence učenja, prilagodljivost in občutljivost sistema ter minimalno potrebno število nevronov za izvedbo določene naloge. Za vsako platformo bomo predlagali izvedljive učne algoritme, pri čemer bomo težili k načinom implementacije sprotnega učenja. To bo v sistemih *in vivo* omogočilo večjo prilagodljivost sistema na spreminjajoča se kompleksna okolja in natančnejše odzivanje na različne vhodne signale. Področje načrtovanja in priprave SYNBIONN zahteva tehten premislek o možnih posledicah, ki bi jih njihova uporaba prinesla. Visoka stopnja prilagodljivosti lahko povzroči nepredvidljivo vedenje, zaradi česar je nujna vključitev nadzornih mehanizmov, ki bodo preprečili preživetje SYNBIONN v primeru neželenega vedenja. Z razvojem te tehnologije je potrebno spodbujati razpravo o namenih uporabe in etičnosti SYNBIONN.

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Rapid Yeast Lipid Quantification: Nile Red Plate Assay & Automated Analysis

Mia ŽGANJAR[1,2*], Gabriela GOGG-FASSOLTER[3], Neža ČADEŽ[2], Klaus NATTER[3], Uroš PETROVIČ[1,2]

[1] Jožef Stefan Institute, Department of Molecular and Biomedical Sciences, Ljubljana, Slovenia

[2] University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

[3] Institute of Molecular Biosciences, NAWI Graz, University of Graz, Graz, Austria

* mia.zganjar@ijs.si

Introduction: Accurate lipid quantification in model budding yeast *Saccharomyces cerevisiae* is crucial for understanding lipid metabolism, homeostasis, and their roles in various cellular functions. In this study, we present a high-throughput Nile red plate assay for rapid lipid quantification in yeast, using 96-well and 384-well formats. Nile red is a lipophilic fluorescent dye that exhibits high sensitivity and specificity towards neutral lipids accumulated in special organelles, called lipid droplets (LD) [1]. Our optimized protocol includes a systematic approach to sample preparation, staining, and fluorescence measurements, ensuring consistent and reliable results across different *S. cerevisiae* strains and experimental conditions. Additionally, we developed a Python3-based tool for automated analysis, data processing and basic assay quality checks. Our Nile red plate assay offers an efficient high-throughput platform for lipid biology research in yeast, enabling the investigation of lipid-related processes, gene function, and characterization of mutants with altered lipid phenotypes.

Methods: For the optimisation, mainly prototrophic *S. cerevisiae* strains were grown in 96-well microtiter plates in lipid inducing N-limiting and lipid non-inducing C-limiting media until the stationary growth phase (72 h) at 30 °C. By using various genetically modified and natural yeast strains, we were able to capture the full range of lipid accumulation, from minimal to maximal levels [2-3]. The fluorescence intensity was measured at excitation wavelength λ = 510 nm and emission wavelength λ = 585 nm, following the addition of 20% DMSO solution, which enhances Nile red's solubility and promotes its interaction with neutral lipids [1]. The lipid quantification plate assay supports both 96-well and 384-well microtiter plates formats, offering increased throughput and flexibility. Optimal signal linearity was determined by testing various Nile red concentrations and biomass volumes. Assay results were validated by comparison with thin-layer chromatography (TLC), confirming accuracy and reliability. The

Python-based command-line tool for automated data analysis reads raw measurement files, excludes outlier measurements, attaches plate layouts with metadata, and calculates lipid content with biomass normalization. The two outputs from the analysis – the outlier-excluded raw data and the final data frame with layouts, metadata, and normalized lipid content – are in .csv format. Additionally, it generates an HTML report containing essential measurement information and quality control metrics.

Results and Conclusions: In this study, we determined optimal conditions for lipid quantification using the Nile red plate assay. Our results highlight the ideal biomass volume and Nile red concentration, along with suitable measurement settings, enabling precise and reliable lipid quantification. A strong correlation (> 0.9) with TLC illustrates our assay as a faster, automatable alternative for lipid quantification, maintaining reliability in measuring yeast intracellular neutral lipids without time-consuming extraction steps. We also evaluated the assay's performance in both 96-well and 384-well formats, revealing consistency and reproducibility regardless of the chosen format. This adaptability, combined with a user-friendly Python-based tool, establishes a high-throughput platform for investigating lipid-related processes, gene function, and characterizing mutants with altered lipid phenotypes. Future work will focus on incorporating statistical testing into the analysis and developing a graphical user interface (GUI) to enhance user-friendliness and analytical capabilities.

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BIOTEHNOLOGIJA

Primarne epitelne celične kulture iz mleka kot model za preučevanje mastitisa

Zala BRAJNIK[1]*, Jernej OGOREVC[1]

[1] Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za zootehniko, Domžale, Slovenija

* zala.brajnik@bf.uni-lj.si

Uvod: Mastitis povzročajo različni patogeni, ki lahko posredno ogrožajo zdravje človeka, vplivajo pa tudi na kakovost mleka in mlečnih izdelkov. Razumevanje molekularnih procesov naravne odpornosti proti mastitisu ter patogen-specifičnih molekularnih interakcij med različnimi povzročitelji bolezni in gostiteljem je ključnega pomena za dolgoročno izboljšanje odpornosti v mlečnih čredah. Patogeni, ki vstopajo v mlečno žlezo preko seskovega kanala, prihajajo v stik z epitelnimi celicami mlečne žleze (MEC), ki imajo poleg sekretorne in drugih funkcij tudi pomembno vlogo v prirojenem imunskem odzivu. Študije na MEC so pokazale prisotnost molekul, povezanih s prirojenim imunskim sistemom, npr. vzorčno prepoznavnih receptorjev in protimikrobnih peptidov, ki so prisotni v mlečni žlezi že pred okužbo. Raziskave na celičnih kulturah mlečne žleze lahko predstavljajo alternativo poskusom na živalih. So cenejša, hitrejša in etično sprejemljivejša alternativa. Vir celic pri vzpostavitvi primarnih celičnih kultur običajno predstavljajo tkiva. Pridobitev donorskega tkiva je pogosto povezana z invazivnimi metodami odvzema (biopsija), poleg tega pa je tkivo po celični sestavi običajno heterogeno, kar otežuje pridobitev homogenih celičnih linij. Znano je, da določen delež somatskih celic v mleku predstavljajo tudi MEC. Možnost pridobitve MEC na neinvaziven način, v našem primeru iz mleka, zato predstavlja prednost in omogoča vzpostavitev homogenih linij MEC, brez potrebe po mehanski in encimatski obdelavi tkiva.

Material in metode: Mleko smo pridobili od krave na začetku laktacije. Primarne epitelne tkivne kulture mlečne žleze smo vzpostavili iz mleka krav po optimizirani metodi Danowski in sod. (2012). Iz mleka smo s centrifugiranjem pridobili pelet celic. Tega smo resuspendirali v segretem (37 °C) pufru za spiranje (HBSS), ki je vseboval dodatek antibiotikov. Resuspendirane celice smo filtrirali skozi 100 µm sito v novo centrifugirko, jih ponovno centrifugirali in resuspendirali v rastnem gojišču z dodatkom antibiotikov. PbMEC smo gojili v skladu z uveljavljeno metodo (Ogorevc in sod., 2009) pri 37 °C, 5 % CO_2 in 100 % vlažnosti. Vzpostavljene primarne celične kulture so pogosto morfološko heterogene (npr. kontaminacije

s fibroblasti), zato smo v primeru kontaminacij, za obogatitev deleža epitelnih celic, izvedli diferenčno tripsinizacijo (izkoriščanje različnih časov odlepljanja različnih celičnih tipov od podlage). Za karakterizacijo celičnih tipov smo poleg preverjanja morfoloških značilnosti uporabili imunofluorescenčno barvanje proti označevalcem, značilnim za epitelne celice mlečne žleze prežvekovalcev. Z barvanjem smo potrdili prisotnost epitelnih celičnih (pod)tipov v vzpostavljeni primarni celični kulturi. Z namenom preučevanja prirojenega imunskega odziva smo pbMEC imunsko stimulirali z virulentnimi dejavniki, npr. lipopolisaharidom (LPS), pridobljenim iz bakterije *E. coli*, ter lipoteihoično kislino (LTA), pridobljeno iz bakterije *S. aureus*.

Rezultati in razprava: Iz vzorcev mleka smo uspeli vzpostaviti primarno celično linijo epitelnih celic kravje mlečne žleze (pbMEC). Celice v kulturi so imele značilno morfologijo epitelnih celic – kompaktni skupki celic poligonalnih oblik, ki so bile trdno pritrjene na podlago. Imunofluorescentno barvanje je potrdilo prisotnost označevalcev, značilnih za epitelne celične tipe. V celični liniji so bile zastopane tako mioepitelne (KRT14+) celice, ki omogočajo krčenje alveol, kot luminalne celice (KRT18+), ki so pomembne za sintezo mleka. Preliminarni rezultati kažejo, da se celice ob stimulaciji z LPS ali LTA odzovejo s povečanim izražanjem provnetnih citokinov (npr. *CCL5*). Stimulacija pbMEC z LPS je povzročila večje število reguliranih genov in večje spremembe v izražanju v primerjavi z LTA. Vendar pa je bilo več pomembnih proinflamatornih citokinov med najbolj reguliranimi v obeh primerih (npr. *CCL5*, *CXCL8*, *IL1B*). Vzpostavitev celične kulture pbMEC iz mleka je bila uspešna, saj so se celice delile in izražale tipične epitelne označevalce. Z imunofluorescenčnim barvanjem smo dokazali, da v kulturi pbMEC prevladujeta dva epitelna celična tipa – luminalni in mioepitelni. Preliminarni rezultati kažejo, da so vzpostavljene pbMEC imunsko odzivne in primerne za nadaljnje raziskave preučevanja okužb mlečne žleze.

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Development of *Lactococcus lactis* strains for potential growth and lactic acid production on cellulosic biomass

Petra ŠTRAVS[1*], Stéphanie PERRET[2, 3], Aleš BERLEC[1, 4]

[1] Jožef Stefan Institute, Department of Biotechnology, Ljubljana, Slovenia

[2] The French National Centre for Scientific Research, Laboratoire de Chimie Bacterienne, Marseille, France

[3] Aix-Marseille University, Marseille, France

[4] Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

* petra.stravs@ijs.si

Introduction: The circular economy and the transition to more sustainable sources of raw materials, biochemicals and their production is a current and important topic being discussed in the framework of the European Green Deal. One of the main goals is to limit greenhouse gas emissions in order to reduce climate change and achieve climate neutrality. To achieve the aforementioned goal, it is crucial to reduce dependence on fossil fuels by developing more sustainable biochemicals that would be competitive in price with petrochemical alternatives. Polylactic acid (PLA) is a good example of a biodegradable polymer and a viable replacement for conventional petroleum-based plastics. PLA is made from lactic acid, which is produced through fermentation of sugars derived from crops that are also used in human and animal nutrition. This makes production of PLA in large quantities and at an affordable price challenging. Plant waste would provide a significantly more sustainable and cost-effective option for the feedstock (substrate). Lactic acid bacteria (LAB) are the most common producers of lactic acid. To promote growth on cellulosic substrates, LAB must be equipped both with extracellular cellulases and transporter systems that enable them to decompose cellulose and uptake the products of the decomposition.

Material and Methods: To develop LAB with cellulolytic capabilities, we used the bacterium *Lactococcus lactis*, the model organism for genetic engineering of LAB. Using classical cloning approaches with restriction enzymes, we prepared a series of plasmid constructs for constitutive expression of three heterologous cellulases derived from three different cellulolytic bacteria. In these constructs, we inserted a secretion signal sequence for secretion of the cellulases, an anchor sequence for surface presentation of the cellulases, and a short tag sequence for detection of the cellulases. We examined the expression and secretion of

cellulases by the obtained *L. lactis* strains in conditioned culture media concentrated with trichloroacetic acid using SDS-PAGE followed by Western blot (WB) analysis. Surface presentation of cellulases was evaluated by dot-blot analysis of *L. lactis* cells. Cellulases were detected with primary antibodies against the tag and fluorescently labelled secondary antibodies. The ability of heterologously expressed cellulases to break down the β-1,4-glycosidic bond in cellulose was assessed on carboxymethylcellulose (CMC) agar plates with Congo red staining. The ability of heterologous cellulases to bind crystalline cellulose was evaluated by incubating conditioned medium with cellulose. The bound and unbound fractions were analysed by SDS-PAGE followed by WB analysis.

Results and Conclusions: We successfully developed *L. lactis* strains expressing all three heterologous cellulases. We confirmed the secretion of all three cellulases and their presence on the cell surface of *L. lactis,* anchored to the cell wall by two different anchors. We also confirmed the activity of all three cellulases on the soluble modified cellulose substrate CMC by the appearance of decolorization zones. The ability to degrade CMC varied among these cellulases. All three cellulases can bind to crystalline cellulose. This binding was very strong for two cellulases, as they were present in the bound fraction only, while the third cellulase was also present in the unbound fraction. To date, our results indicate that functional cellulases can be expressed, secreted, and displayed on the cell surface of *L. lactis*. This work lays the ground for the creation of genetically engineered *L. lactis* strains that can utilise plant waste for growth and lactic acid production.

HORTIKULTURA

Preventive and curative effects of salicylic and methyl-salicylic acid against *Monilinia laxa* and the metabolic response of apple peel

Saša GAČNIK[1*], Alenka MUNDA, Robert VEBERIČ[1], Metka HUDINA[1] in Maja MIKULIČ-PETKOVŠEK[1]

[1] University of Ljubljana, Biotehnical faculty, Department of Agronomy, Ljubljana, Slovenia

* sasa.gacnik@bf.uni-lj.si

Introduction: Food production is facing the challenge of producing enough quality food in the face of climate changes, the resistance of harmful organisms to chemical agents, and the desire of consumers for healthy food produced with the least possible phytopharmaceutical products (Babalar et al., 2007; Calzadilla et al., 2013; Droby et al., 2009). New environmentally friendly approaches are needed to protect plants from biotic and abiotic stress factors (Tyagi et al., 2022). One of the possible alternatives could be salicylic acid (SA), which is a phenolic compound present in plants (Raskin, 1992) that has an important influence on plant development, physiological processes, and plant adaptations to stress (Klessig & Malamy, 1994). Activation of the SA synthesis pathway is usually triggered by pest attack or pathogen infection, but can also be stimulated by exogenous use of elicitors (Filgueiras et al., 2019). The exogenous use of SA has been shown to be effective against several diseases, including anthracnose (Zainuri et al., 2001), cherry brown rot (Yao & Tian, 2005), apple scab, brown leaf spot (Abbasi et al., 2019), potato late blight (Halim et al., 2007) and apple blue mold (da Rocha Neto et al., 2016).

Material and Methods: This study was performed on the apple cultivar 'Golden Delicious', at the Biotechnical Faculty in Ljubljana, considering three factors – infection (infected (INF) or uninfected (unINF), solution use (preventively (PRE)/curatively (CUR)) and solution type (SA/Methyl salicylic acid (MeSA); water (C)). To test PRE, apples were dipped in a 2.5 mM SA or MeSA for 30 min. Apples from all INF treatments were then infected through a puncture wound, through which a suspension of *M. laxa* spores were applied. Apples from all treatments were then incubated for 24 h in a growth chamber at 20 °C and 100% humidity. After 24 hours, apples from CUR treatment were immersed in a 2.5 mM SA or MeSA for 30 min and returned to the growth chamber. The intensity of infection, the average lesion growth rate (LGR; mm/day) and disease incidence (%) was first assessed after 3 days (D1). On the sampling date

D5, healthy tissue and boundary section of the lesion were removed from the apples with a 1–2 mm narrow strip of healthy tissue for further HPLC-MS (High-performance liquid chromatography- Mass spectrometry) analysis of the phenolic compounds content (mg kg^{-1} FW), which was performed as described by Gacnik et al. (2023).

Results and Conclusions: CUR use of 2.5 mM SA and MeSA slowed the progression of brown rot infection. On the other hand, there were no significant differences between PRE and the control. This may be because of the low persistence of SA and MeSA in apple peel after the application. Analysis of the content of phenolic compounds by HPLC-MS, detected phytoanticipins, which in most cases increased in the boundary tissue around the infection point on the peel, as well as flavanols, hydroxycinnamic acids and dihydrochalcones On the contrary, flavonol content was higher in the healthy tissue of the infected fruits. We hypothesize that dihydrochalcones and flavanols elicited a stronger phenolic response. The use of salicylates decisively increased the content of flavanols, hydroxycinnamic acids and dihydrochalcones in the boundary tissue around the lesion, whereas in healthy peel tissue only CUR treatments affected the contents of flavonols, flavanols, hydroxycinnamic acids and total analyzed phenolics in infected fruit.

Kinetics of thermal degradation of ellagitannins and total phenolics in strawberry nectar of cultivar 'Senga Sengana' (*Fragaria* × *ananassa*)

Alema PUZOVIĆ[1*], Maja MIKULIČ-PETKOVŠEK[1]

[1]University of Ljubljana, Biotechnical Faculty, Agronomy Department, Ljubljana, Slovenia

*Alema.Puzovic@bf.uni-lj.si

Introduction: Berry products contain nutrients and phytochemical compounds, that are beneficial for human health due to their antioxidant properties, such as ellagitannins which have positive effects on many pathologies. However, in strawberries some of them are vulnerable to thermal processing, such as pigments and phenolic compounds, hence, it is necessary to assess kinetic changes in these bioactive components during the juice/nectar production process to determine optimum processing conditions while preserving nutrient content. Therefore, the aim of this study is to evaluate the effect of different time - and temperature combinations on ellagitannins and total phenolic concentration and on their degradation rate in strawberry nectar made from *Fragaria* × *ananassa* 'Senga Sengana' cultivar.

Material and Methods: 1 kg of puree from strawberry *Fragaria* × *ananassa* cultivar 'Senga Sengana', along with 2.81 g of citric acid, 230 g of sucrose and 1.27 l of tap water was used in order to produce 2.5 l of strawberry nectar with the following characteristics: dry substance 12° Bx, fruit portion 40 %, pH value 3.1-3.5. Nectar processing parameters included different temperature (T) / time (t) combinations; time: 0 – 5 – 10 – 15 – 20 – 25 – 30 min, temperature: 70 – 80 – 90 – 95 °C. Processing was done in 3 replicates for each T/t combination, resulting in 84 samples. The phenolic compounds identification was performed using a HPLC-MS system according to Mikulič-Petkovšek et al. (2014) and the total phenolic content (TPC) was assessed by the Folin-Ciocalteau phenol reagent method.

Results and conclusions: With an average concentration of 36.9 mg/l, total ellagitannins accounted for cca. 5.4% of the total share and were the third most abundant group of phenolic compounds, after anthocyanins and hydroxycinnamic acid derivatives that accounted for 41.1% and 40.6%, respectively. The highest concentration of ellagitannins was obtained at 80°C with heat treatment duration of 15 minutes. However, with higher temperatures (90 and 95°C) and longer duration of heat period (15-30 min), total ellagitannins concentration significantly decreased. Total phenolic compounds, with an average concentration of 684.9

mg/L, were most stable at 80°C, decreasing slightly with longer heat periods (20-30 min), but with no significant differences between treatments. By calculating the activation energy (the minimum energy that molecules must possess in order to react) during the same period of time, the decline in total ellagitannins and phenolic compound concentration occurred significantly faster and in greater amounts at higher temperatures (90 and 95°C) compared to lower ones (70 and 80°C). These results imply that the ideal T/t combination is 75°C/15 mins and 75°C/20 mins, which can ensure that the nectar is pasteurized and has high concentration of ellagitannins and total phenolic compounds.

Genomic analysis of ESBL-producing *Escherichia coli* from bloodstream infections

Leon MARIČ[1,2]*, Helena RIBIČ[1], Urška KRAMAR[1], Andrej Golle[1], Maja Rupnik[1,3], Sandra JANEŽIČ[1,3]

[1] National Laboratory of Health, Environment and Food, Center for Medical Microbiology, Department for Microbiology Research, Maribor, Slovenia

[2] University of Ljubljana, Biotechnical faculty, Department of Microbiology, Ljubljana, Slovenia

[3] University of Maribor, Faculty of Medicine, Maribor, Slovenia

*leon.maric@nlzoh.si

Introduction: Extended-spectrum beta-lactamases (ESBL) producing bacteria *Escherichia coli* is, according to World Health Organisation (WHO), one of the critical priorities among important antibiotic-resistant pathogens and is also a major cause of nosocomial outbreaks. Surveillance of bacterial infections and nosocomial outbreaks is critical for identifying and controlling the spread of infectious diseases in healthcare settings. Whole-genome sequencing (WGS) has revolutionized how bacterial outbreaks are detected and investigated. It provides detailed information about genetic relatedness and other characteristics such as virulence factors, antimicrobial resistance genes, and horizontal gene transfer. Our research aimed to utilize WGS and bioinformatic analyses to determine the relatedness of ESBL-producing *E. coli* isolated from humans. Furthermore, we have analysed the presence of genes and plasmids associated with antimicrobial resistance (AMR), which can help us to understand the antimicrobial resistance mechanisms in different isolates.

Material and Methods: Forty-two clinical isolates from human blood samples obtained in the year 2021 were subjected to whole genome sequence analysis. The isolates were from a large Slovenian diagnostic laboratory, mostly from elderly patients. After the extraction of DNA, whole genome sequencing was performed on Illumina short-read platform, PE150 (Illumina NextSeq2000). With a built pipeline for quality control analysis, we filtered out low-quality reads, adapters, and other artifacts that could affect downstream analyses. For each *de-novo* assembled genome, a core genome multilocus sequence typing (cgMLST) was performed using the Warwick scheme and Ridom SeqSphere+ software. For a more detailed comparison, we

also performed analysis of single nucleotide polymorphism analysis (SNP). SNP analysis included mapping reads on reference genome (NC_002695.2), generating consensus sequences using BWA, BCFtools and Samtools, variant calling, core genome alignment using Snippy, and removing recombination sites using Gubbins. The presence of antimicrobial resistance genes was determined using different tools and databases (AMRFinder, ResFinder, and CARD). For plasmid detection and reconstruction, we utilize PlasmidFinder and MOB-suite tools.

Results and Conclusions: All 42 ESBL-producing *E. coli* strains were assigned to 6 different MLST-STs, with 32 (78%) of the strains belonging to ST131. Subsequent cgMLST analysis did not confirm the clonal spread. Clonal clusters, which included isolates that differed from each other by no more than ten alleles, were identified only among 12 ST131 strains. One cluster consisted of four isolates, while the remaining four clusters each consisted of two isolates. The clonal isolates were predominantly obtained from a single hospital, except for two clonal isolates that were found in both hospitals. SNP analysis confirmed the results of cgMLST analysis and revealed the same relatedness between isolates. Most of the ESBL-producing *E. coli* harboured $bla_{CTX\text{-}M\text{-}15}$ (n=29), but $bla_{CTX\text{-}M\text{-}27}$, $bla_{CTX\text{-}M\text{-}3}$, $bla_{CTX\text{-}M\text{-}1}$, $bla_{OXA\text{-}1}$, $bla_{TEM\text{-}1}$, and bla_{TEM} were also detected. Isolates belonging to ST131, carried either $bla_{CTX\text{-}M\text{-}15}$ (n=23) or $bla_{CTX\text{-}M\text{-}27}$ (n=8). One ST131 isolate harboured $bla_{CTX\text{-}M\text{-}3}$. Beta-lactamases were found to be mostly located on plasmids, highlighting the importance of horizontal gene transfer. All beta-lactamases were located on IncFIA, IncFIB, IncFIC, IncFII, or IncI1/B/O plasmids. Beta-lactamases $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}3}$ were also located on IncI-gamma/K1 and IncL/M plasmids, respectively.

ZNANOST O CELICI

The underlying mechanisms of prolonged membrane depolarization following electroporation are temperature dependent

Anja BLAŽIČ[1*], Lea REMS[1]

[1] University of Ljubljana, Faculty of electrical engineering, Ljubljana, Slovenia

* anja.blazic@fe.uni-lj.si

Introduction: All cells maintain an electric potential difference across their plasma membranes, which results from the differences in membrane permeabilities for potassium, sodium, calcium, and chloride ions and is maintained by a system of ion channels and pumps. This electric potential difference is called the resting transmembrane voltage or resting membrane potential. By convention the resting transmembrane voltage is negative, meaning that the cell interior is electrically more negative compared to its exterior. When cells are electroporated (exposed to intense pulsed electric field), the permeability of the membrane to ions and other molecules transiently increases in nonselective manner, and their transmembrane voltage remains depolarized for several minutes after pulse exposure. The aim of our study is to better understand the mechanisms of prolonged membrane depolarization following electroporation.

Materials and Methods: U-87 MG human glioblastoma and Chinese hamster ovary (CHO) cells were plated in Nunc Lab-Tek II chambered coverglass three to four days before the experiments. For monitoring changes in transmembrane voltage, the cells were stained for 30 min at 37 □C with the Component A of the FLIPR Membrane Potential Assay Red, which was diluted in Live Cell Imaging Solution. For monitoring the kinetics in propidium iodide (PI) uptake (to assess the increase in plasma membrane permeability due to electroporation), PI (30 µM) was added in Live Cell Imaging Solution to cells 5 minutes before pulse delivery. Cells were exposed to a single 100 □s, 1.4 kV/cm pulse under two temperature conditions – uncontrolled (ambient temperature) and controlled temperature (set at 37 □C, with the temperature in the sample stabilized at 33 ˚C). Pulses were delivered by Electrocell B10 pulse generator through a pair of Pt-Ir wire electrodes. Time lapse images of the cells were acquired before and after pulse application. The cells were imaged on inverted microscope Leica DMi8 with LED8 illumination source controlled by the LasX software.

Results and Conclusions: By studying two different cell lines, i.e. CHO and U-87 MG, stained with a potentiometric dye, we show that depolarization depends on the temperature. Not only do the cells remain depolarized for a longer period of time when electroporated at uncontrolled temperature, but they are also often slightly depolarized even before electroporation, with higher and more scattered initial fluorescence values compared to controlled temperature conditions (33˚C). While the depolarization depends on temperature, the kinetics of propidium iodide uptake does not depend on temperature. The results of our study suggest that prolonged changes in transmembrane voltage happening minutes after the pulse delivery cannot be simply attributed to nonselective leak current due to membrane permeabilization and that its mechanisms are more complex. Since the transmembrane voltage has an important biological function by controlling the activity of various membrane proteins, its changes can influence the response of cells to electroporation. This may be an important consideration in various electroporation applications, including gene therapy, tumor treatment and cardiac ablation.

Vloga plektina pri razporejanju citoskeleta in migracijah astrocitov

Maja ŽUGEC[1]*, Maja POTOKAR[1,2]*

[1] Univerza v Ljubljani, Medicinska fakulteta, Inštitut za patofiziologijo, Laboratorij za endokrinologijo – molekularna celična fiziologija, Ljubljana, Slovenija

[2] Celica Biomedical, Ljubljana, Slovenija

* maja.zugec@mf.uni-lj.si, maja.potokar@mf.uni-lj.si

Uvod: Astrociti, ena najštevilčnejših skupin celic v centralnem živčnem sistemu, so svoje ime dobili leta 1891, ko jih je na podlagi njihove zvezdaste oblike poimenoval Michael von Lenhossek. Astrociti so dolgo veljali za celice, ki imajo pasivno vlogo zagotavljanja podpore nevronom, vendar danes vemo, da opravljajo številne naloge in da nepravilnosti v njihovem delovanju lahko vodijo v različne patologije. V embrionalnem razvoju astrociti migrirajo vzdolž radialnih glialnih celic, kar je ključen proces pri razvoju možganov, njihove migracije pa se v zgodnjem postnatalnem obdobju zaključijo. Astrociti v fizioloških pogojih v možganih odraslih migrirajo le izjemoma, in sicer v nekaterih primerih vzdrževanja homeostaze in morfogeneze tkiv. V patoloških razmerah, kot so okužbe, travma, ishemija in nevrodegenerativne bolezni, pa so migracije astrocitov pomembne predvsem za nastanek astrogliozne brazgotine, prispevajo pa tudi k invazivnosti tumorjev. Migracije celic omogoča citoskelet, predvsem mikrotubuli (MT) in aktinski filamenti (AF). Prerazporejanje citoskeleta je uravnavano preko adaptorskih in povezovalnih proteinov. Plektin spada v družino plakinov, velikih, večdomenskih proteinov, ki povezujejo elemente citoskeleta med seboj in s s proteinskimi kompleksi pomembnimi za tvorbo stikov med sosednjimi celicami in z zunajceličnim matriksom. Te povezave so ključnega pomena za vzdrževanje mehanskih lastnosti celic in tkiv. Mutacije genov, ki kodirajo plakine so povezane z različnimi boleznimi. Plektin je edini plakin, ki se izraža v astrocitih in je pomemben za povezovanje AF, MT in intermediarnih filamentov.

Material in metode: Imortalizirane mišje astrocite smo pridobili z Univerze na Dunaju, kjer so bili izolirani iz neonatalnih $Plec^{-/-}p53^{-/-}$ in $Plec^{+/+}p53^{-/-}$ miši, pridobljenih s križanjem $Plec^{+/-}p53^{+/-}$ heterozigotnih miši. Za namen poskusov smo celice v primerni gostoti nasadili na krovnike s poli-D-lizinom. AF smo označili z uporabo reagenta Phalloidin-iFluor 594 (Abcam, Velika Britanija), MT pa s primarnimi protitelesi proti α-tubulinu in s sekundarnimi protitelesi, konjugiranimi z AlexaFluor 546. Slike preparatov smo zajeli s konfokalnim mikroskopom

Zeiss LSM 800, z uporabo oljnega imerzijskega objektiva 63 ×/NA 1,40. Za analizo migracij smo celice nasadili na dvodelne petrijevke s pregrado (Ibidi GmbH, Nemčija), ki jo po 24 urah odstranili. Z mikroskopom Zeiss LSM 800, z uporabo objektiva 10 ×/0,45 smo v štiri-urnih intervalih beležili premike celic. Za analizo razporeditve citoskeleta (vzporednost filamentov citoskeleta z najdaljšo osjo celice in kopičenje filamentov citoskeleta t.j. združevanje v skupke) in za analizo migracij astrocitov smo uporabili program Fiji (ImageJ). Statistično analizo smo izvedli v programu SigmaPlot 11.0 (SYSTAT, ZDA), za statistično značilne razlike smo upoštevali vrednosti $P < 0,05$.

Rezultati in razprava: Rezultati so pokazali, da odsotnost plektina v *plec*$^{-/-}$*p53*$^{-/-}$ astrocitih povzroči prerazporeditev AF stran od plazmaleme, hkrati pa so AF tudi bolj vzporedni in nakopičeni, kot v *plec*$^{+/+}$*p53*$^{-/-}$ astrocitih. MT pa so v odsotnosti plektina v *plec*$^{-/-}$*p53*$^{-/-}$ astrocitih razporejeni bližje plazmalemi, so pa, podobno kot AF, razporejeni bolj vzporedno, kot so v *plec*$^{+/+}$*p53*$^{-/-}$ astrocitih. Rezultati analize migracij so pokazali, da imortalizirani astrociti, ki ne izražajo plektina, migrirajo počasneje od tistih, ki plektin izražajo. Povzeto, rezultati kažejo, da je vpliv plektina na organizacijo in razporeditev elementov citoskeleta različen za posamezen tip citoskeleta. Odsotnost plektina upočasni hitrost kolektivnih migracij astrocitov, kar kaže na možno vlogo plektina pri razvoju in poteku patoloških stanj, kjer je stopnja izražanja plektina spremenjena in kjer so migracije astrocitov ponovno vzpostavljene.

ŽIVILSTVO

Evaluation of foodborne diseases databases

Maja BENSA[1*], Mojca JEVŠNIK[2]

[1] University of Ljubljana, Faculty of Health Sciences, Research Institute of Faculty of Health Sciences, Ljubljana, Slovenia

[2] University of Ljubljana, Faculty of Health Sciences, Department of Sanitary Engineering, Ljubljana, Slovenia

[*] maja.bensa@zf.uni-lj.si

Introduction: Food safety has significantly improved in recent years, yet foodborne diseases remain a global problem. Every year foodborne diseases cause nearly one in 10 people to become ill after consuming contaminated food. According to the World Health Organization every year hundreds of thousands of people die due to foodborne diseases (WHO, 2022). Therefore, information about outbreaks of foodborne disease is of high importance for experts working in the field of food safety, since the data can be used to evaluate the established protocols, to observe trends, to develop solutions to improve food safety as well as to decrease the occurrence of outbreaks. Different countries have different approaches to management of foodborne disease information. The aim of this study was to compare and evaluate three databases (two from EU and one from US) for their information relevant to food safety experts. Databases evaluated in this study included: 1) Foodborne outbreaks - dashboard (FBO of the European Food Safety Authority) (EFSA, n.d.), 2) Surveillance Atlas of Infectious Diseases (ATLAS of the European Centre for Disease Prevention and Control) (ECDC, n.d.) and 3) National Outbreak Reporting System Dashboard (NORS of the Centers for Disease Control and Prevention, United States) (CDC, 2022).

Material and Methods: The evaluation of three databases (FBO, ATLAS and NORS) was performed in terms of their user interfaces, limitations and their possible roles in analysis and further development of food safety policies. The following technical properties were evaluated: layout, filtering, graphics (such as maps, graphs, tables), statistical overviews and options for data export, etc.

Results and Conclusions: The evaluation revealed that FBO, ATLAS, and NORS are very useful databases with a high potential for assisting food safety experts. Each of the three databases offers a different user interface and does not include all the same categories of data

(e.g., location of outbreaks). The NORS dashboard has a more user-friendly interface than FBO and ATLAS. The data in all three databases can be used to evaluate and improve food safety policies.

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