https://doi.org/10.18524/2077-1746.2024.2(55).320491

БІОЛОГІЧНА СЕКЦІЯ (23 СЕРПНЯ 2024 р.) В МЕЖАХ XXIV ГАМОВСЬКОЇ МІЖНАРОДНОЇ АСТРОНОМІЧНОЇ КОНФЕРЕНЦІЇ «АСТРОНОМІЯ ТА НЕ ТІЛЬКИ: АСТРОФІЗИКА, КОСМОЛОГІЯ ТА ГРАВІТАЦІЯ, АСТРОФІЗИКА ЕЛЕМЕНТАРНИХ ЧАСТИНОК, РАДІОАСТРОНОМІЯ, АСТРОБІОЛОГІЯ ТА ГЕНЕТИКА», ЯКА ПРОВОДИЛАСЬ 19–23 СЕРПНЯ 2024 р. В ОНУ ІМЕНІ І. І. МЕЧНИКОВА, ОДЕСА, УКРАЇНА

23 серпня 2024 року вже вшосте в межах роботи Гамовської міжнародної астрономічної конференції відбулося засідання Біологічної секції. Біологічна секція традиційно проходила під назвою «Важливість ідей Г. Гамова для біології 21 століття». Цього року у роботі секції взяли участь 39 науковців та студентів. Свої наукові доповіді представили вчені з України, Німеччини, Великобританії, Данії, Канади та США. Доповіді охоплювали дослідження, що базуються на вивченні нуклеотидних послідовностей генів та геномів різних біологічних видів.

Ключові слова: Біологічна секція, Гамовська міжнародна астрономічна конференція, нуклеотидні послідовності, ген, геном.

THE BIOLOGICAL SECTION (23 AUGUST 2024) WITHIN THE XXIV GAMOW INTERNATIONAL ASTRONOMICAL CONFERENCE “ASTRONOMY AND BEYOND: ASTROPHYSICS, COSMOLOGY AND GRAVITATION, ASTROPARTICLE PHYSICS, RADIO ASTRONOMY, ASTROBIOLOGY AND GENETICS”, HELD ON 19–23 AUGUST 2024 AT ODESA I. I. MECHNIKOV NATIONAL UNIVERSITY, ODESA CITY, UKRAINE

It was the sixth session of the Biological Section within the Gamow International Astronomical Conference that took place on 23 August 2024. The Biological Section was traditionally held under the title “Importance of G. Gamov’s Ideas for the Biology of the 21st Century.” This year, 39 scientists and students participated in the work of the section. Scientists from Ukraine, Germany, Great Britain, Denmark, Canada and the USA presented their scientific reports. Those reports covered research based on the studies of nucleotide sequences of genes and genomes of various biological species.

Keywords: Biological Section, Gamow International Astronomical Conference, nucleotide sequences, gene, genome.

Професор С. Чеботар, керівник секції, відкрила засідання промовою про внесок Георгія Антоновича Гамова у розвиток молекулярної біології. Вона нагадала, що на сьогоднішній день написано низку книг, присвячених Г. А. Гамову та його ролі у розшифруванні генетичного коду. Серед них книга Джеймса Д. Вотсона, лауреата Нобелівської премії та першовідкривача подвійної спіралі ДНК, «Genes, Girls and Gamow» (2001); книга професора Пенсильванського університету Джино Сегре «Ordinary Geniuses: How Two Mavericks Shaped Modern Science» (2011), присвячена Максу Дельбрюку та Георгію Гамову, які в певний час товаришували та обговорювали наукові ідеї визначення генетичного коду (ця книга була перевидана у Києві українською мовою у 2017 році), а також книга Гораса Фріланда Джудсона «The Eighth Day of Creation: Makers of the Revolution in Biology» (1996). Цього року Біологічна секція включала широкий спектр наукових доповідей, що підкреслюють важливість ідей Георгія Гамова для сучасної біології.

Під час роботи секції було представлено 14 наукових доповідей. Доктор біологічних наук М. Борисюк з Інституту клітинної біології та генетичної інженерії НАН (м. Київ) представив результати досліджень, виконаних спільно з науковцями Huaiyin Normal University (Китай), у доповіді «First nucleotide level structure of 5S and 35S rDNA loci for plants». Дослідження генів, що кодують рибосомні РНК, було висвітлено у доповіді професора Р. Волкова та його співавторів «Conserved sequence elements in the 5S rDNA intergenic spacer are present in the genomes of distantly related Angiosperms»; ці дослідження були виконані на кафедрі біотехнології і генетики Чернівецького університету (м. Чернівці).

Молода науковиця Ю. Попович у доповіді «Application of new molecular markers for detecting genetic polymorphism of Ukrainian cultivars of Triticum durum Desf.» представила результати досліджень, проведених під керівництвом професора С. Чеботар на кафедрі молекулярної біології, біохімії та генетики ОНУ імені І. І. Мечникова. Також в доповіді були представлені результати досліджень, проведених у співпраці з науковцями Селекційно-генетичного інституту – Національного центру насіннєзнавства та сортовивчення (м. Одеса).

На особливу увагу заслуговувала демонстрація практичного застосування CRISPR-Cas9 технології для створення гаплопродьюсорів у ячменю – цю роботу представила аспірантка P. Satpathy у доповіді «Generation of haploidy inducers in barley by Cas9-mediated knockout of phospholipase A1». Її робота, виконана під керівництвом професора J. Kumlehn (Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Гатерслебен, Німеччина), викликала велику зацікавленість серед вітчизняних біотехнологів, зокрема О. Л. Шестопал і І. С Замбріборщ, які працюють у Селекційно-генетичному інституті – Національному центрі насіннєзнавства та сортовивчення.

Про важливість забезпечення принципів FAIR для дослідницьких даних, а саме доступності та повторного використання накопичених даних у міжнародних проектах програми Horizon 2020 говорила Г. Чеботар у своїй доповіді

«Ensuring data accessibility and reusability: the FAIR approach to research data management», що висвітлює роботу з даними, які було отримано в межах наукового проекту INCREASE, виконаного у Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Гатерслебен, Німеччина.

Цілу низку надзвичайно цікавих досліджень з генетики бактерій представили науковці Львівського національного університету імені Івана Франка. Серед них варто відзначити доповідь О. Ющука «Developing a genetic toolkit to manipulate the rare actinobacterium *Umezawaea endophytica*» та доповідь аспірантки С. Мельник «Exploring the genomic potential of *Streptomyces roseochromogenes* NRRL 3504 for specialized metabolite production». Також із доповідями виступили аспіранти В.-М. Цедуляк «Effects of mutated ribosomal protein S12 on *Streptomyces Albidoflavus* J1074» та І. Роман «The impact of bioinformatics tools in the classification of prokaryotes: the case of the genus *Actinoplanes*». Науковиця з Копенгагенського університету й Орхуського університету (Данія) Д. Жарікова у своїй доповіді «The unexplored relationship between the epigenome and the root microbiome of *Zea maize*» привернула увагу до необхідності досліджувати взаємодію між кореневим мікробіомом та рослинами.

За напрямом молекулярно-генетичних досліджень людини були висвітлені сучасні підходи до діагностики та лікування. Так, доповідь науковиці із St George's University of London та генетика St George's University Hospitals Dr. K. Snape «Cancer genomics: clinical interrogation of the two genomes of cancer patients» привернула велику увагу і продемонструвала переваги застосування ДНК-технологій у діагностичних цілях. Від української корпорації «Юрія-Фарм» Олександр Губар представив доповідь «Multifactorial engineering of capless mRNAs for enhanced in vitro potencies», присвячену використанню РНК-технологій у лікуванні.

Дослідження із застосуванням молекулярно-генетичних технологій, що проводяться в McGill University Health Center (м. Монреаль, Канада), були висвітлені у доповіді Dr. Yu. Monczak «Measurable residual disease (MRD) in lymphoid malignancies: the haystack, the needle, and how to find it».

Наприкінці роботи Біологічної секції, Dr. X. Li з Вашингтонського державного університету (м. Вашингтон, США) представив доповідь «Graphing large indel-based haplotypes from pan-genome facilitates gene discovery», присвячену застосуванню пангеноміки для вивчення гаплотипів та їхньої ролі у відкритті нових генів.

Попри складні умови воєнного часу, перерви електроживлення та часові відмінності через географічні локації доповідачів, науковці-біологи провели дуже продуктивну сесію й висловили щирі сподівання на перемогу України у війні та настання миру!

UDC 58:577.213/.215

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FIRST NUCLEOTIDE LEVEL STRUCTURE OF 5S AND 35S rDNA LOCI FOR PLANTS

Ribosomal DNA (rDNA) plays an important part in eukaryotes by encoding four ribosomal RNAs necessary for ribosome biogenesis, thus regulating organism's growth and development. Plants typically have two types of rDNA loci: the 5S rDNA loci, containing tandem copies of 5S rRNA genes separated by non-transcribed spacers (NTS), and the 35S rDNA loci, encoding three linked rRNA genes (18S-5.8S-25S) intertwined with intergenic spacers (IGSs) [1]. Taking advantage of low copy number of rDNA repeats in aquatic monocot plant *Spirodela polyrhiza* [2] (about 100 copies of both 5S and 35S rDNA genes, compared to thousands copies in most of plant species), we revealed the detailed molecular organization of the rDNA loci combining molecular cytology, conventional and extra-long Oxford Nanopore (ON) sequencing. First, we show that relatively G/C rich 35S rDNA and 5S rDNA arrays are imbedded in highly A/T-enriched chromosome regions. Cloning and nucleotide sequencing of the 35S rDNA repeats located in a single chromosome locus, demonstrated high conservation of the sequences encoding 18S, 5.8S, and 25S rRNAs and some intra-genomic heterogeneity in IGSs with an unorthodox structure of usually conserved rRNA transcription initiation site (TIS). The latter finding is intriguing, considering that TIS in the related species of *Pistia stratiotes* [3] has a canonic TATAGGGG signature typical for majority of the plants species. Validation of rough ON data by sequencing multiple plasmids with PCR fragments covering individual 5S rDNA units, demonstrated that the 5S rDNA repeat clusters localize on two different chromosomal loci, which was further confirmed by *in situ* hybridization. The loci are correspondingly composed of 40 and 60 repeat units containing the NTSs of different size and demonstrating different rates of sequence variability, further supporting the contrasting evolutional dynamics of the two types of 5S rDNA units in *S. polyrhiza* [4]. In summary, our findings put duckweeds in the spotlight for research on the molecular evolution of the rDNA, promising new insights into basic principles of organization and regulation of rRNA genes in plants.

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UDC 577.113 + 582.5

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CONSERVED SEQUENCE ELEMENTS IN THE IGS 5S rDNA OF ANGIOSPERMS

Tandemly arranged repetitive regions (repeats) that encode 5S rRNA (5S rDNA) are an obligatory component of eukaryotic genomes. It is widely accepted that 5S rDNA repeats within a genome are very similar due to the mechanisms of concerted evolution. Each 5S rDNA repeat consists of a conserved coding sequence (CDS) and a rapidly evolving intergenic spacer (IGS). The high evolutionary stability of the CDS is the result of purifying selection to maintain the function of the 5S rRNA as a component of the ribosome. In contrast, it is thought that the main part of the IGS probably has no function and therefore evolves at a high rate.

In this work, we aim to uncover the patterns of IGS evolution in a broad taxonomic context. We applied cloning, Sanger sequencing, and bioinformatic analysis of whole-genome Illumina sequencing data to describe the molecular organization of 5S rDNA in 31 genera representing 15 families and 12 orders of angiosperms.

It was shown that the short sequence motifs at the 5’ and 3’ ends of the IGS, which are involved in the initiation and termination of 5S rDNA transcription, evolve relatively slowly and are obviously conserved within a genus/family. Unexpectedly, our analysis also detected conserved sequence elements (CSEs) with a length of 50 to 200 bp in the middle part of the IGS in representatives of eight eudicot genera. These CSEs show significant similarity (70 to 91%) to genomic sequences from taxonomically distant families. For instance, CSEs from the 5S rDNA IGS of *Lens*

spp. (Fabaceae) were found in the genomes of members of the families Malvaceae, Oleaceae and Salicaceae. Similarly, CSEs in the IGS of *Aconitum*, *Delphinium* and *Anemone* species (Ranunculaceae) matched sequences in the genomes of Oleaceae and Amaryllidaceae. Notably, sequence similarities to CSEs were found only in the 5S rDNA IGS of some *Allium* (Amaryllidaceae) species. Finally, CSEs from the IGS of *Lycium*, *Nicotiana* and *Petunia* (Solanaceae) showed similarity to genomic sequences of Fagaceae, Linaceae, and Malvaceae.

We suggest that the presence of similar sequence elements in the genomes of distantly related taxa may be due to putative horizontal DNA transfer. Alternatively, the CSEs could represent ancient sequences conserved in certain phylogenetic lineages. The function of the CSEs and the mechanisms responsible for their maintenance during long-term evolution remain enigmatic and require further investigation.

UDC 606:632]:633.16

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GENERATION OF HAPLOIDY INDUCERS IN BARLEY BY CAS9-MEDIATED KNOCKOUT OF *PHOSPHOLIPASE A1*

The use of doubled haploid (DH) lines is one of the most effective biotechnological measures in modern plant breeding. Individual DH lines are genetically unique results of meiotic recombination, while they are possessing the important characteristic of being entirely true-breeding. This means that once a useful DH line is selected, it can be identically reproduced through selfing. In barley, DH lines can be efficiently obtained via microspore-derived plant regeneration. However, this principle is genotype-dependent to some extent. An alternative means to produce DH lines is to employ haploidy-inducing lines as paternal parents (Satpathy *et al*. 2021). Among the progeny resulting from such crosses, maternal haploids can be found that have lost the paternal genome during early embryogenesis. The phenomenon of uniparental genome elimination was reported to occur in mutants including those for *Centromeric histone 3* (*CenH3*), *Phospholipase* (*PLA1*, *PLD3*) as well as *DUF 679 membrane protein* (*DMP*) genes of various species (Liu *et al*. 2017). Primary barley mutants carrying Cas9-triggered mutations in *PLA1* produced about 6% haploid progeny upon pollination of wild-type plants. The haploidy-inducing capacity of homozygous *pla1* M2 mutants was then validated by pollination of various barley accessions, which resulted in haploid formation from 6% to 16%. In

a further approach, we are employing *cas9/*gRNA-transgenic *pla1* mutant barley to deliver these transgenes and their respective products from sperm cells to zygotes via fertilization. Any maternal parents of choice may thus be subjected to genome editing, while the transgene-carrying paternal genome is expected to getting lost in some cases during embryo formation (Budhagtapalli *et al.* 2020). This concept holds great promise for barley genome editing with considerably reduced genotype dependency.

Acknowledgments

We thank Sabine Sommerfeld, Nicole Schäfer, Carola Bollmann and Ingrid Otto for excellent technical support, also the team of Enk Geyer for taking care on the plants in the glasshouse. This work was partially funded by the German Federal Ministry of Education and Research (DELITE project, FKZ 031B0550).

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UDC 575.17:575.113.2:633.34

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APPLICATION OF NEW MOLECULAR MARKERS FOR DETECTING OF GLIADIN LOCI POLIMORPHISM OF *TRITICUM DURUM* DESF.

Durum wheat is an important raw material in the food processing industry and the main ingredient in the production of pasta. It is mainly grown in the Mediterranean basin (Italy, Turkey, Algeria and Spain, providing 50% of the world's production).

Usually durum wheat cultivars with extraordinary frost tolerance have unsatisfactory yield and quality. Breeding winter –type cultivars of durum wheat with

frost tolerance and quality on a level of classical quality spring durum varieties and yield is challenging, but not impossible. Flour breadmaking/ pastamaking quality is determined by properties of gluten complex consisting of monomeric gliadins and polymeric glutenins proteins.

Gliadins and glutenins demonstrate high polymorphism and are very important for selection durum wheat especially of winter type cultivars selection, growing in Ukraine. Due to the complexity identification allelic variants of gliadins in durum wheat there is a need for DNA-markers. Therefore, the aim of study was to analyze polymorphism of *Gli-A1* and *Gli-B1* loci of modern Ukrainian winter durum wheat cultivars using a DNA-marker system developed on common wheat.

Eighteen durum wheat cultivars developed by Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigations were analyzed by PCR with allele-specific primers to *Gli-A1* and *Gli-B1* loci, developed by Zhang et al (2003). Gliadin protein specters characteristics were revealed by storage proteins electrophoresis in acid PAGE method. Bioinformatic analysis and PCR *in silico* were used for analyzing possibility of applying of primers developed for bread wheat for durum wheat.

By using PCR with allele-specific primers to *Gli-A1* locus *Gli-A1.2* allele was detected in all cultivars studied. For six cultivars two alleles *Gli-A1.1* and *Gli-A1.2* alleles revealed in each grain analyzed. We assume these cultivars could have two copies of amplified in PCR sequence, that was shown by bioinformatic analysis.

Using PCR with allele-specific primers to *Gli-B1* locus developed by Zhang, we revealed two diferent by length amplification fragments of *Gli-B1.1* allele: 376 bp and 379 bp. Also five different by length amplification fragments were detected for *Gli-B1.2* alele: 397 bp, 400 bp, 403 bp, 409 bp, 424 bp.

DNA-marker system for gliadins developed on common wheat (Popovych et al., 2020) is applicable for durum wheat. Most alleles detected in the study are common for *T. durum* and *T. aestivum* species. Allele frequencies revealed for durum wheat significantly differ from allele frequencies of bread wheat collections studied before.

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UDC 606:631.528:004

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ENSURING DATA ACCESSIBILITY AND REUSABILITY: THE FAIR APPROACH TO RESEARCH DATA MANAGEMENT

The application of multi-omics approaches for crop investigations presents researchers with challenges related to the storage and integration of the obtained data. Over the last few decades, the amount of data generated through various international and collaborative projects has risen dramatically, and this trend is expected to continue in the future. Therefore, clear strategies for data management and reusability are essential. Data may be consistent with FAIR principles: Findable, Accessible, Interoperable and Reusable, but still may be as open as possible and as closed as necessary. Effective research data management strategies include the publication of data in repositories with persistent identifiers, regular backups, detailed descriptions of data formats, and comprehensive metadata documentation.

An example of open science, open innovation and open to the world project handling large volumes of data is the INCREASE project, funded by Horizon 2020. This six-year initiative involves 26 partners from 13 countries, focusing on the food legumes common bean, chickpea, lentil, and lupin (https://www.pulsesincrease.eu/about). The project aims to develop efficient conservation tools to enhance agricultural biodiversity in Europe. From a data curation perspective, the goal is to ensure meaningful and enduring access to the generated data.

For approximately 18,000 accessions, single seed descendant lines, around 13,000 persistent identifiers have been created (e.g. https://doi.org/10.18730/13MRAB), making the data findable and accessible online. Standardized templates have been developed for about 50 traits across four crops to gather data from 80 experiments, each with three repetitions. Data quality is evaluated based on metrics such as correctness, completeness, fitness for use, and alignment with project objectives. Passport data provided by project partners are standardized to MCPD (FAO/Bioversity Multi-Crop Passport Descriptors V.2.1 [MCPD V.2.1] https://www.fao.org/plant-treaty/tools/toolbox-for-sustainable-use/details/en/c/1367915) and matched with EURISCO and GENESYS. For phenotypic data an ISA-TAB like (https://www.nature.com/documents/scidata-isatab-specification.pdf) format is applied. Further data base implementation and web portal development are expected. Information from genotyping and metabolomic experiments and results of data analysis will be published in open repositories, e.g. Zenodo, e!DAL, ENA.

In addition to laboratory research, the project includes a Citizen Science Experiment, allowing volunteers to participate in decentralized seed conservation and agro-biodiversity preservation. Data collected through the Citizen Science App are also integrated into the project's database.

UDC 575+579

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DEVELOPING A GENETIC TOOLKIT TO MANIPULATE THE RARE ACTINOBACTERIUM *UMEZAWAEA ENDOPHYTICA* DSM 103496

Mycelial actinobacteria – colloquially known as actinomycetes – are soil-dwelling Gram-positive bacteria with a high genomic GC content. Several features of actinomycetes attract scientific attention, both in fundamental and applied contexts. Fundamentally, these organisms are studied as models of bacterial multicellularity due to their complex life cycles involving cellular differentiation. On the applied side, actinomycetes are of interest for their production of various specialized metabolites, including antibiotics, and biotechnologically relevant enzymes. Historically, members of the genus *Streptomyces* have been the most extensively studied actinomycetes, providing the majority of antibiotics of microbial origin. However, members of other genera (often referred to as non-*Streptomyces* actinobacteria or non-common actinomycetes) are equally interesting and prolific sources of specialized metabolites, despite being much more challenging to cultivate, handle, or manipulate genetically.

In our previous research aimed at discovering novel glycopeptide antibiotics (GPAs – a valuable group of compounds that inhibit Gram-positive cell wall biosynthesis by binding to lipid II), we identified a biosynthetic gene cluster (BGC) for a putative new GPA in the genome of the non-common actinomycete *Umezawaea endophytica* DSM 103496 (family *Pseudonocardiaceae*). With the ultimate goal of identifying the production of this novel GPA, we first decided to thoroughly investigate the growth properties of the strain and develop a toolkit for genetic manipulations with DSM 103496.

When the growth and morphology of DSM 103496 were studied on various agar media, we discovered that the culture actually consisted of two distinct morphological types. The first, named Bht (from “barchent,” due to its velvety surface), exhibited typical *Pseudonocardiaceae* morphology, including the development of aerial mycelium that differentiated into spores, as evident from scanning electron

microscopy (SEM) imaging. The second type, named Lth (from "leathery," due to its smooth surface), did not form aerial mycelium or spores. SEM imaging revealed that the Lth type had a smooth surface composed of vegetative hyphae with short, spike-like, presumably hyphal structures that did not resemble aerial hyphae. These hyphal structures were easily fragmented and dissociated when the lawn surfaces were washed with water.

After analyzing several thousand clones of Bht and Lth, no transitions between the two morphological types were observed, confirming the stability of these forms. Notably, co-cultivation of Lth and Bht induced the formation of typical aerial hyphae in Bht, suggesting that Bht is unable to produce some small-molecule messenger required for proper differentiation (similar to the A-factor of *Streptomyces* spp.).

Next, we tested whether hyphal fragments (Lth) and spores (Bht) could serve as recipients in intergeneric conjugation with *Escherichia coli* donor strains carrying various integrative and replicative plasmids. We found that φC31-, φBT1-, and VWB-actinophage-based plasmids, as well as a pSG5-replicon-based replicative plasmid, could be successfully transferred using hyphal fragments of Lth. However, none of these plasmids could be transferred when spores of Bht were used as recipients.

Overall, the obtained results highlight DSM 103496 as a peculiar example of a non-common actinomycete. We plan to sequence the genomes of both Bht and Lth to identify genetic differences underlying their distinct morphologies and further optimize conjugation protocols for both forms. These findings represent an initial step toward investigating GPA production in DSM 103496.

UDC 575+579

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EXPLORING THE GENOMIC POTENTIAL OF *STREPTOMYCES ROSEOCHROMOGENES* NRRL 3504 FOR SPECIALIZED METABOLITE PRODUCTION

The current crisis of antibiotic therapy in Ukraine and the world forces us to look for new approaches to discovering antibiotic compounds. The explosion of bacterial genome data has led to the discovery of myriads of specialized metabolite biosynthetic gene clusters (BGCs) of unknown chemical identity. Developing reliable methods to access the chemical diversity hidden in these predominantly "silent" BGCs will help

discover new biologically active compounds. Here, we report the use of a complex approach combining detailed bioinformatic analysis of the genome of soil bacteria *Streptomyces roseochromogenes* NRRL 3504 with further expression of identified BGCs in suitable host strains.

NRRL 3504 was first described in 1970s as a producer of gyrase inhibitor clorobiocin and no other specialized metabolites are known to be produced by this strain till this report. We conducted thorough bioinformatic analysis of NRRL 3504 genome to predict chemical identity of small molecules this strain could produce and genetic mechanisms that may limit their production. As a result we managed to express in the heterologous hosts *S.albus* Del14 and *S. lividans* ΔYA9 several BGCs putatively involved in biosynthesis of anti-inflammatory antibiotic colabomycin E, antitumor agent trioxacarcin A and cancer cells inhibitor ulleungdin. In-depth analysis of colabomycin-like BGC (hereafter labeled as *lim*) revealed that the latter in fact directs the production of unusual polyketide limocrocin (LIM) known for its antiviral activities. Bioinformatic analysis of *lim* BGC as well as *lim* gene knockouts led to the initial hypothesis about biosynthetic pathway leading to LIM that remained hidden for the last 70 years.

UDC 575+579

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EFFECTS OF MUTATED RIBOSOMAL PROTEIN S12 ON *STREPTOMYCES ALBIDOFLAVUS* J1074

The discovery of spontaneous streptomycin-resistance mutations in ribosomal protein S12 gene (*rpsL*) that influence translational speed and accuracy has revealed that the ribosome is more than a just passive platform for protein biosynthesis. Some *rpsL* mutations induce mistranslation, while others promote slow and hyperaccurate protein synthesis. Beyond their direct impact on translation, *rpsL* mutations exert pleiotropic effects on important antibiotic-producing *Streptomyces* bacteria, including upregulation of specialized metabolites production, aberrant protein biosynthesis, and increased expression of ribosome recycling factor.

Previous studies of *rpsL* mutations were performed on spontaneous mutants carrying additional changes in their genomes. In contrast, our study focuses on genetically engineered *S. albidoflavus* mutants K88R, K88E, and R94G which provide valuable insights into *rpsL*-mediated effects due to their ‘clean’ genetic background.

These three strains, differing only in one amino acid residue within S12 protein, exhibit altered morphology features: the wild-type strain SAM2 and R94G show unchanged appearance, while K88E and K88R have a reduced number of sporulating hyphae. Ribosomal protein S12 mutations did not adversely affect total protein content or colony-forming unit counts under the conditions tested, but mutants' dry biomass weight was significantly decreased compared to the wild-type strain. Moreover, the level of c-di-GMP, a second messenger regulating morphological development and antibiotic biosynthesis, was affected in the mutants, particularly in K88R, which was extremely difficult to generate. The highest antibioticproduction levels were observed for K88E and R94G strains exhibiting moderately decreased c-di-GMP levels.

To conclude, our data suggest that certain *rpsL* mutations affect c-di-GMP levels, through as-yet unknown mechanism. A better understanding of this phenomenon can shed light on the antibiotic overproduction phenotype commonly associated with *rpsL* mutations. This will guide rational ways of *Streptomyces* strain improvement, urgently needed in times rising of antimicrobial resistance .

UDK 579.873.8

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THE IMPACT OF BIOINFORMATICS TOOLS IN THE CLASSIFICATION OF PROKARYOTES, THE CASE OF THE GENUS *ACTINOPLANES*

During the Antarctic rhizosphere actinobiome research, about 100 isolates were isolated. Based on the results of 16S rRNA gene sequencing, members of nine genera were identified, including four isolates from the genus *Actinoplanes*. Since the members of the genus *Actinoplanes* were isolated from Galindez Island for the first time, we decided to perform a detailed phylogenetic analysis of these isolates. The 16S rRNA gene sequences of 57 validly published species of the genus *Actinoplanes* were selected for this work, with *Pseudosporangium* as an outgroup. However, during the phylogenetic analysis, *Pseudosporangium* was grouped with certain species of the genus *Actinoplanes* and formed a separate clade. Therefore, it was decided to perform a phylogenetic analysis of the entire *Micromonosporaceae* family to better calibrate the phylogenetic tree. However, the obtained results were a bit unexpected; the genus *Actinoplanes* was not only grouped with other genera (*Pseudosporangium*,

Couchioplanes, *Mangrovihabitans*, *Nucisporomicrobium* and *Jidongwangia*), but also formed three almost independent clades. Thus, two hypotheses emerged: 1) the genera *Pseudosporangium*, *Couchioplanes*, *Nucisporomicrobium* and *Jidongwangia* are described incorrectly and should actually belong to the genus *Actinoplanes*; 2) the genus *Actinoplanes* is actually heterogeneous and includes several separate genera that are mistakenly included in *Actinoplanes*. For further work, we divided the genus *Actinoplanes* into 4 clades and studied their properties independently. After performing the ANI analysis, we were able to establish that the members of each studied clade have high similarity within the clade, but outside of it, the similarity drops rapidly and does not significantly differ from the similarity to the members of other genera. In addition, the ANI also allowed us to divide one of clades into two subclades, as their representatives were highly diverse. The results were also confirmed by AAI analysis. As a result, four new genera were described: *Paractinoplanes*, *Winogradskya*, *Symbioplanes* and *Amorphoplanes.*

UDC 575.113:582.3/.99

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THE UNEXPLORED RELATIONSHIP BETWEEN THE EPIGENOME AND THE ROOT MICROBIOME

The relationship between the microbiome and the plant is a complex and dynamic one, with bidirectional interactions that can have significant impacts on the plant's fitness [1, 2]. Mechanisms involved in microbiome regulation through genetic factors of the host plant and the properties of the plant microbiome are well documented [3, 4]. However, the plant genome is vital but may not fully explain plant functions due to environmental influences [5]. In response to the external stresses epigenetic changes can impact gene expression without altering the DNA sequence, via DNA methylation, histone modifications, and small RNA-mediated gene silencing. Aimed to afford a more comprehensive understanding of how the plant responds to environmental cues and how it shapes the composition and activity of its associated microbiome, along with host genome here we propose to contemplate epigenome influence.

Here, performing two experiments in greenhouses, we focused on the influence of the plant epigenome on the root microbiome. Experiment 1 showed that a mutation affecting the epigenome led to changes in the root microbiome, potentially due to alterations in root morphology and exudates. Experiment 2 demonstrated differences in the root microbiome of plants from parents exposed to phosphorus stress,

attributing this variation mainly to inherited epigenetic differences rather than seed endophytes. While epigenome effects were inconsistent in phosphorus samples and absent in nitrogen samples, genomic variation greatly affects the epigenome-microbiome relationship. The findings suggest that variations in the epigenome can have significant effects in real-world scenarios beyond controlled environments. This highlights the need for further research with replicates and clonal plant populations to better understand the complex relationship between the epigenome and the root microbiome.

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UDC 575.111:616-006.6

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CANCER GENOMICS: CLINICAL INTERROGATION OF THE TWO GENOMES OF CANCER PATIENTS

Cancer patients have two clinically relevant genomes. Firstly, the somatic (acquired) cancer genome which contains genomic variation leading to oncogenesis. This cancer genome can be interrogated for prognostic, diagnostic or therapeutic information to guide cancer management. Secondly, the constitutional (germline) genome. This contains heritable genomic variation which can increase the likelihood of a person developing cancer and can be utilised to identify patients at increased risk of cancer who might benefit from additional screening, prevention or early detection interventions.

In the UK, paired whole genome sequencing in cancer patients took place initially through the 100,000 Genomes research study and is now embedded within the National Health Service (NHS), alongside large cancer gene panel next generation sequencing as part of the routine care of cancer patients.

In this lecture, I will give an overview of the clinically relevant genomic variation which can be identified through analysis of these two genomes in cancer patients, using case exemplars to demonstrate important principles and how our ability to generate large scale genomic data is transforming the care of cancer patients in clinical practice.

UDC 577.213/.216:577.113

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MULTIFACTORIAL ENGINEERING OF CAP-LESS MRNAS FOR ENHANCED IN VITRO POTENCIES

Cap-less mRNAs represent an emerging platform for therapeutic nucleic acids developed by Yuria-Pharm LLC, seeking to address key limitations of traditional cap-dependent mRNA manufacturing, such as high reagent costs, complex purification steps, and suboptimal product yields. These novel mRNAs incorporate engineered group I-like lariat-capping ribozymes (LCRs) to generate a stable 5′-cap structure and employ an internal ribosome entry site (IRES) for cap-independent translation initiation.

In this study our primary goals were: (I) to identify LCR variants with increased 5′-end capping efficiency and downstream protein expression, and (II) to identify 3′UTR elements that enhance transcript stability in cells. A panel of natural LCR orthologs from protists [1,2], as well as site-directed mutants of the *Didymium iridis* LCR, was generated and tested *in vitro*. We identified variants exhibiting up to a three-fold increase in processing rates and improved capping efficacy (from 45% to 93%), highlighting the importance of rapid lariat product release after the transesterification step. When incorporated into synthetic mRNAs carrying an IRES, these optimized LCRs produced a four-fold increase in luciferase output relative to the IRES-only control, while reaching up to 20% of reporter accumulation, driven by ARCA-capped benchmarks. LCR performance differed significantly depending on the IRES sequence context, underscoring mutual dependence on the sequence/folding context for both elements.

To further improve mRNA stability, we performed functional screening of a 3′UTR library[3] derived from a pool of long-lived transcripts isolated from CHO DG44 cells after prolonged cell culture incubation with Actinomycin D. By subjecting this library to iterative selection (six rounds of transfection, extended

incubation, re-cloning and mRNA library re-synthesis), we identified 194 candidate 3′UTR features showing significant enrichment, with 89 variants displaying over ten-fold enrichment relative to the initial library. Over-representation analysis of enriched features demonstrated enrichment of multiple GO terms including positive regulators of transcription, transcripts associated with rRNA metabolic processes and regulators of translation, typically associated with processes, demanding for long-lived transcripts. 26 putative 3′UTRs were selected for further validation in reporter assays.

Our work demonstrates that rational engineering of LCR in tandem with careful selection of 3′UTRs can substantially improve both capping efficiency and functional stability of cap-less mRNAs, accelerating the clinical translation of cap-less mRNA therapeutics.

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UDC 616-006.04

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MEASURABLE RESIDUAL DISEASE (MRD) IN LYMPHOID MALIGNANCIES: THE HAYSTACK, THE NEEDLE, AND HOW TO FIND IT

Most B- and T-cell lymphoid malignancies develop from a single cell by acquiring a set of mutations in various genes, each providing a specific growth advantage to that transformed cell. Since the immunoglobulin heavy (IGH) chain genes rearrange very early in lymphoid cell differentiation, most clonal populations of lymphocytes will carry the exact same gene rearrangement as in the original malignant cell. Moreover, the IGH gene rearrangements are very stable throughout clonal expansion, hence they can be used as clonal identity markers. Molecular detection of lymphoid malignancies (B- or T-cell) has quickly become the gold-standard in diagnosis, and regular end-point PCR technology using the BIOMED-2 protocol has been the “gold standard” for over twenty years [1]. It relies on the identification of the specific

IGH gene rearrangement common to the clonal population of cells. More recently, next-generation sequencing (NGS) has established itself as the method of choice for detection of lymphoid clonality, providing higher sensitivity and specificity, and allowing additional downstream evaluation of disease treatment and response [2].

One such evaluation is the detection of the "tumor burden" in the patient following treatment, referred to as Measurable Residual Disease (MRD) [3]. Recent studies suggest that patient progression-free survival can be predicted depending on the number of remaining tumor cells, usually measured by the number of circulating clonal cells divided by the number of polyclonal (normal) cells in a patient's blood sample [4]. The target value is at least $1:10^4$, and prognosis improves if the MRD value reaches $1:10^5$ or even $1:10^6$ [5]. Although flow-cytometry provides reliable MRD analysis at least up to $1:10^4$, NGS technology allows us to rapidly determine the exact sequence of the clonal IGH gene rearrangement in the malignant cell population at diagnosis, and then use this sequence to calculate the number of times it is found in a pool of the patient's cells post-therapy, with MRD values up to $1:10^6$. The cornerstone of this methodology is the quantity of lymphocytes that must be sequenced in order to provide the necessary "depth of sensitivity". In order to achieve a sensitivity of $1:10^4$, a total of 200ng of DNA must be sequenced, which translates to 30,000 cells. If one is to achieve a sensitivity of $1:10^6$, then over 3 million cells need to be sequenced, which poses a challenge for sample sizes and method costs. Here we describe our attempts to achieve such high levels of sensitivity.

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UDC 575.113

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GRAPHING LARGE INDEL-BASES HAPLOTYPES FROM PAN-GENOME FACILITATES GENE DISCOVERY

Large insertion and deletion (indel) polymorphisms contribute significantly to phenotypic variations through altering gene structure or expression. Although pan-genome assemblies are available from major crops, identifying and graphing large indels across assemblies for specific genes are challenging and painstaking tasks.

To overcome the challenge, we devised two unsupervised learning algorithms, CHOICE (Clustering HSPs for Ortholog Identification via Coordinates and Equivalence) and CLIPS (Clustering via Large-Indel Permuted Slopes). CHOICE automatically retrieves the segments harbouring the ortholog from each assembly for the desired All-vs-All comparison while CLIPS groups accessions sharing same indels into haplotypes for concisely haplotype graphing.

We then constructed an interactive webapp BRIDGEcereal (https://bridgecereal.scinet.usda.gov/) to expedite this process. Over hundred assemblies from 5 major cereal crops (Wheat, Barley, maize, rice, and sorghum), were compiled. The only required input is a gene model ID or a transcript sequence. Two adjustable parameters, up- and down-stream search boundaries, enable to survey the unknown sizes and locations for indels outside of the gene body. We demonstrated that mining pan-genome through BRIDGEcereal could accelerate gene discovery and characterization with multiple wheat genes underlying QTL/GWAS intervals.

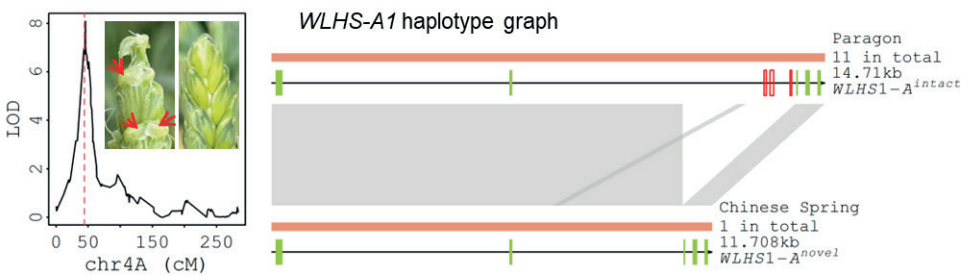

Fig. 1. *The haplotype graph generated by BRIDGEcereal suggests WLHS-A1, a MADS-box gene segregating a 3-kb indel containing 3 exons (open red boxes), is a promising candidate for the classic Hooded QTL.*

The gene underlying the *B1* locus controlling awn presence/absence had been identified as a novel transcription factor while the functional site remains unknown. BRIDGEcereal identified large deletions of 17-kb in the *B1* upstream contributing to two haplotypes as potential casual polymorphisms. *Hooded* is another QTL controlling awn presence/absence, but the underlying gene had not been identified. Results from BRIDGEcereal suggested that *WLHS-A1* harbouring a 3-kb deletion removing 3-exons as a promising gene. The versatile design enables to seamlessly incorporate newly released assemblies.

Acknowledgments

USDA-ARS In-House Project (2090-21000-033-00D), PDI, SciNet, and Iowa State University Crop Bioengineering Center seed grant.

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