Abstract for Oral Presentation

O-01. On the Hunt: Characterizing Candidate Orphan Genes from the Dark Genome

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Abstract

A substantial portion of bacterial genomes remain poorly characterized, forming part of the microbial "dark genome." This includes hypothetical genes and proteins predicted computationally but lacking experimental validation. Candidate Orphan Genes (COGs) are especially enigmatic because they lack detectable homologs outside specific taxonomic groups, limiting functional prediction through traditional homology-based approaches. In our previous study, we reassessed bacterial COGs and developed a computational framework to identify candidates with the strongest evidence for encoding protein products. Building on this work, our broader objective is to determine experimentally whether these sequences represent bona fide protein-coding genes and to identify their biological functions. As an initial step towards this goal, we examined transcriptional evidence for the COG, BSU_9999 in *Bacillus subtilis* 168. Raw RNA-sequencing data from 10 independent NCBI BioProjects were analyzed to assess expression, transcriptome relative abundance, and condition-dependent changes. BSU_9999 was expressed in all datasets and fell within the central 95% of transcriptome-wide expression. The strongest response occurred in minimal medium, with ~14-fold higher expression than in rich medium. These findings support BSU_9999 as a transcriptionally active gene rather than an annotation artifact. Current work focuses on RT-qPCR validation of BSU_9999 expression and generation of a BSU_9999 knockout strain for functional characterization. This integrated genomic, transcriptomic, and experimental framework provides a scalable strategy to distinguish bona fide genes from annotation artifacts and assign functions to poorly characterized genes within the bacterial dark genome. BSU_9999 serves as an initial test case for an approach that can be expanded to additional COGs.

O-02. ReoGenotyper: an alignment-free open-set neural classifier for avian orthoreovirus segment genotypes and ten-segment constellations

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Abstract

Avian orthoreovirus (ARV) has ten double-stranded RNA genome segments with distinct evolutionary histories, yet routine molecular classification remains centered on the σC-encoding region of S1. Single-segment typing cannot describe the composition of a reassorting genome, and phylogeny-based classification does not provide a rapid, stable prediction system for newly submitted sequences. We therefore propose an operational ARV genotype constellation in the fixed order L1–L2–L3–M1–M2–M3–S1–S2–S3–S4 and implement it in ReoGenotyper. To our knowledge, ReoGenotyper is the first openly distributed, end-to-end machine-learning package that identifies an ARV segment, assigns segment-specific genotypes across the complete genome, rejects unsupported inputs, and assembles

a ten-segment constellation. The reference system was constructed from 630 coding-complete chicken and turkey genomes. Each segment was aligned with MAFFT, pairwise nucleotide identity was calculated over unambiguous sites, and average-linkage clustering produced stable operational labels. S1/σC was prioritized for its high variability and established discriminatory value in chicken ARV, whereas M2/μB provided complementary resolution among turkey ARV lineages. These segments were classified at 75% identity, whereas the other eight segments used 85%. The final reference contained 14 S1 genotypes, four M2 genotypes, three to five genotypes for each remaining segment, and 61 complete constellations. ReoGenotyper learns canonical reverse-complement-invariant k-mer patterns with a segment multilayer perceptron and ten genotype-specific multilayer perceptrons. Prototype-distance checks add an open-set decision so that an unrelated virus, wrong segment, or insufficiently supported genotype is not forced into a known class. Connected components sharing at least 99% nucleotide identity were kept within one partition during model evaluation. Of 1,236 similarity-group-disjoint held-out ARV sequences, 1,232 received the correct segment assignment and 1,189 received an accepted genotype. Every accepted genotype matched its reference label, with segment-specific genotype coverage ranging from 90.5% to 100%. All 20 held-out unrelated-virus controls and all 53 sequences in an independent challenge panel were rejected. ReoGenotyper converts a complete-genome ARV classification into an installable prediction system and provides a reproducible foundation that can be expanded through versioned reference releases as new sequence diversity becomes available.

O-03. Development of a multiplex digital PCR approach to simultaneously screen for five distinct stool methanogen species

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Abstract

Intestinal methanogens play important roles in host health and hydrogen balance, yet traditional diagnostic tools like breath methane testing and 16S rRNA gene sequencing lack high-throughput efficiency and absolute species-level resolution. We developed and validated a multiplex digital PCR (dPCR) assay targeting the *mcrA* gene for rapid, simultaneous detection and absolute quantification of five human gut methanogen species: *Methanobrevibacter smithii*, *Methanobrevibacter intestini*, *Methanobrevibacter oralis*, *Methanobrevibacter arboriphilus*, and *Methanosphaera stadtmanae*.

Following reaction optimization (incorporating Q-Solution and temperature gradient fine-tuning), the 5-plex dPCR panel was evaluated across 229 human stool samples from a microbiome registry cohort, comprising healthy controls (n=47), patients with pulmonary arterial hypertension (PAH; n=140), and patients with inflammatory bowel disease (IBD; n = 42). Results were validated against 16S rRNA gene amplicon sequencing.

Methanogens were detected in 31.0% (71/229) of all samples by dPCR. Species prevalence was highest in healthy controls (38.3%), followed by PAH (36.4%) and IBD (4.9%). *Mbr. smithii* was the dominant species (73.3% of positive samples), followed by *Mbr. intestini* (26.7%), *Msp. stadtmanae* (9.9%), *Mbr. arboriphilus* (1.4%), and *Mbr. oralis* (1.4%). Total methanogen absolute copy numbers ranged up to >10^5 copies/µL and strongly correlated with 16S rRNA *Methanobrevibacter* relative abundance ($r=0.78$, $p<0.0001$).

This 5-plex dPCR assay provides a sensitive, reproducible, and rapid platform for species-specific quantification of human gut methanogens. This tool enables high-throughput investigation into the roles of individual archaeal species in gastrointestinal health, metabolic status, and systemic disease.

O-04. Evaluation of a Chicken-Origin Avian Metapneumovirus Subtype B Vaccines in Turkeys

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Abstract

Avian metapneumovirus remains a threat to the poultry industry. Vaccination is one of the best control measures. We have developed a live-attenuated through the frequent passage of U.S. strain-chicken origin (aMPV-B/chicken/USA/SD-24/P6) in Vero cells and an inactivated vaccines against avian metapneumovirus subtype B (aMPV-B). Both vaccines demonstrated significant safety and efficacy in chickens. Here, we evaluated these vaccines in turkeys, the heterologous species of origin. A total of 160 one-day-old commercial turkey poults were divided into four equal groups (n=40). The first group remained unvaccinated. The second group was vaccinated at one day of age with the live-attenuated vaccine via the oculonasal route. The third group received the live-attenuated vaccine at one day of age via the oculonasal route, followed by a booster with the inactivated aMPV-B vaccine at 7 days of age intramuscularly. The fourth group was vaccinated only with the inactivated aMPV-B vaccine via intramuscular injection at 7 days of age. Choanal cleft swabs and blood samples were tested using ELISA and the serum neutralization test. At 24 days of age, poults from each group were challenged with 200 µl of the virulent aMPV-B strain SD-24/B-P6 and monitored for clinical signs and choanal swabs collection. At three, five, and seven DPC, poults from each group were euthanized. The findings confirmed the safety and efficacy of the developed vaccine, with live attenuated being more significant than inactivated alone. The cross-species protection of the developed aMPV-B vaccine could provide a proper vaccine candidate for both chickens and turkeys.

O-05. Advancing the understanding of *Pasteurellaceae* as core members of the livestock vaginal microbiota.

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Abstract

Efficient reproduction is vital for sustainable livestock operations. Despite this, reproductive failure continues to burden producers, leading to economic losses. Recently, the microbial dynamics of the livestock reproductive tract have attracted attention, with evidence that the composition and structure of the vaginal microbiota are linked to conception rates, spontaneous abortion, and postpartum infection. We previously demonstrated that members of the *Pasteurellaceae* family are core inhabitants of the vaginal microbiota of sheep, cattle, and pigs. While certain *Pasteurellaceae* are well documented in the context of disease, the knowledge about their role as commensals in the reproductive tract remains limited. The objective of this study was to isolate *Pasteurellaceae* from the vaginal tracts of livestock to characterize their genetic potential in relation to animal reproductive health using long-read whole-genome sequencing. This effort yielded 27 vaginal strains, including *Histophilus somni*, *Pasteurella mairii*, *Actinobacillus seminis*, *A. rossii*, and *Gallibacterium faecale*. Genomic assemblies ranged from 2.0 to 2.8 mbps and showed evidence of horizontal gene transfer. Notably, type V secretion systems occupied a proportionally large share of the genomes. This included *ibpA* and *ibpB* homologs in *H. somni* genomes, along with other two-partner secretion machinery containing putative contact-dependent inhibition domains. We also identified autotransporter proteins with YadA membrane anchor domains, ranging from 237 to 5,831

amino acid residues. This work addresses the current knowledge gap surrounding *Pasteurellaceae* within the livestock reproductive tract. Current efforts focus on using a bovine endometrial cell culture model to further elucidate the effects of *H. somni* on host reproduction.

O-06. Development and Validation of a Recombinant G Protein-Based Indirect ELISA for Detection of Avian Metapneumovirus Subtype B Antibodies

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Abstract

Avian metapneumovirus subtype B (aMPV-B) has recently emerged in U.S. poultry, highlighting the need for reliable serological tools for diagnosis, surveillance, and vaccine evaluation. This study developed and validated an indirect enzyme-linked immunosorbent assay (iELISA) using recombinant G protein from a U.S. aMPV-B strain. The codon-optimized full-length G gene was expressed in *Escherichia coli*, and the recombinant protein was purified by Ni-NTA affinity chromatography and confirmed by Western blotting. Assay conditions were optimized by checkerboard titration. Performance was evaluated using 1,070 serum samples, including 740 positive and 330 negative samples. Diagnostic sensitivity and specificity were determined by receiver operating characteristic analysis, and assay performance was compared with a commercial APV antibody ELISA. Analytical specificity was assessed using sera positive for other avian pathogens. At an S/P cutoff of 0.35, the iELISA achieved 96.3% sensitivity and 95.6% specificity. Antibody titers strongly correlated with those obtained using the commercial ELISA (Spearman's $\rho = 0.955$, $p < 0.001$). No cross-reactivity was observed with sera positive for avian reovirus, infectious bronchitis virus, infectious bursal disease virus, Newcastle disease virus, turkey arthritis reovirus, hemorrhagic enteritis virus, *Bordetella avium*, *Ornithobacterium rhinotracheale*, or aMPV subtype C. The assay detected vaccine-induced seroconversion by 7 days post-vaccination, with increasing antibody responses through 21 days. These findings demonstrate that the recombinant aMPV-B G protein-based iELISA is a sensitive and specific serological tool for antibody detection and vaccine-response evaluation in poultry.

O-07. The Safety and Efficacy of Recombinant Baculovirus-HA Vaccine Against Low-Pathogenic Avian Influenza (H4N6) in Turkeys

Noreen Bashir, Mohamed Selim, Khalid Abosria, Steven Lawson, Kada Parfait, Ravi Gupta, Kenth Maquiling, Marc Aucoin, Sunil Mor, Tamer A. Sharafeldin*

Abstract

The baculovirus expression system is a safe vaccine delivery platform approved by the USDA for use in livestock. In this study, a unique construct design enabled the expression of the HA (hemagglutinin) gene of low pathogenic avian influenza (H4N6) on the surface of baculovirus. The Spodoptera frugiperda 9 (Sf9) cell line was used to grow and titrate the recombinant baculovirus-HA vaccine. A pilot study using 40 1-day-old turkey poults was run to establish preliminary safety and efficacy. Significant reductions in the gross and histopathology lesion scores of collected samples were seen in the vaccinated-challenged group (V+CG) compared to the non-challenged control group (CCG), suggesting the efficacy of the vaccine. Additionally, the Hemagglutination Inhibition (HI) assay showed a significantly higher antibody titer in the CCG as compared to the V+CG, indicating that the vaccine interfered with the replication of the virus. Viral shedding in the oropharyngeal swabs at 7 days post-challenge was quantitatively measured using real-time RT-PCR. The average viral copy number is remarkably lower in numerical value in the V+CG compared to the CCG, but without a statistically significant difference. This study indicates that HA surface expression on the

baculovirus system could be a safe and protective recombinant vaccine against LPAI-H4N6 in turkeys. Future studies with a larger sample size and other diseases will help prove the concept of recombinant baculovirus vaccine safety and efficacy in poultry.

O-08. Enrichment of prairie soil bacteria growing under nitrogen-deficient conditions

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Abstract

Soil harbors diverse free-living diazotrophs, but its physicochemical and biological complexity limit controlled isolation. Nitrogen-free media are used to isolate diazotrophs, but these media can also support some non-fixers and may miss less abundant diazotrophs. As an alternative approach we enriched diazotrophs using N-free Artificial Soil Microcosms (ASMs). Soil from undisturbed prairies was incorporated into ASMs, supplemented with root exudate cocktail (REC) with and without ammonia. Enrichment was repeated for three cycles. Culturable bacterial density was quantified, and the 16S rRNA gene pool was sequenced to characterize community composition. Single isolates were obtained by end point dilution, followed by genome sequencing. To determine isolates could grow independently in ASM-N, washed cultures were inoculated. To examine whether isolates capable of growing independently in ASM-N could provide nitrogen to isolates unable to grow in ASM-N, we performed co-culture experiments. Culturable counts indicated microbial growth in ASMs, demonstrating active growth under both -N and +N conditions. There are profound shifts in microbial community composition. After four enrichment cycle, the dominant species were *Azotobacter chroococcum, Tolumonas auensis*, and so on. We recovered 35 isolates with similar or distinct morphological characteristics, of which 10 encoded *nifHDKENB* genes, including *A. chroococcum*, *K. variicola* and members of a hitherto undescribed species. Some isolates lacked *nifHDKENB* genes but still grew well under ASM-N conditions. Co-culture experiments showed that *nifHDKENB* $^{+}$ strains support the growth of some *nifHDKENB* $^{-}$ strains. Future work will evaluate whether *nifHDKENB* $^{+}$ isolates can support plant growth under nitrogen-deficient conditions.

O-09. Tracking the tissue distribution of the emergent North American Avian Metapneumovirus Subtypes A and B in turkeys by In-situ Hybridization

Khaled Abosria, Dallas Clontz, Mohamed Selim, Noreen Bashir, David Knudsen, Frederic Hoerr, Sunil Mor, and Tamer A. Sharafeldin

Abstract

The emergence of Avian metapneumovirus (AMPV) subgroup A and B in the USA imposes a great threat to chicken and turkey industry and resulted in substantial economic losses. In 2024, the overall economic impact approached $1.0 billion due to its high morbidity and mortalities as well as reduction in egg production. Clinically affected birds commonly exhibit severe respiratory disease characterized by rhinitis, conjunctivitis, and swelling of the infraorbital sinuses.

This study evaluated the tissue distribution and tropism of AMPV subgroups A and B in naturally infected turkeys using RNA in situ hybridization (ISH). Two RNAscope probes targeting conserved regions of the AMPV nucleoprotein (N) gene were designed to detect and localize viral RNA within tissues. Tissue samples from birds confirmed positive for AMPV by qRT-PCR were examined by ISH. Strong viral RNA signals were detected in the mucosal epithelium of the nasal cavity, infraorbital sinuses, nasal turbinates, conjunctiva, trachea, and

secondary bronchi. In contrast, no detectable signal was observed in the oviduct, testes, brain, or other evaluated tissues.
These findings demonstrate that AMPV subgroups A and B predominantly target the upper respiratory epithelium and support respiratory secretions as an important source of viral shedding under field conditions. The results further indicate that nasal secretions and upper-respiratory mucosal swabs are the most appropriate clinical specimens for detection of AMPV RNA in naturally infected turkeys.

O-10. Not Left To Chance: Soil Health Drives Deterministic Root Microbiome Recruitment Across Crop Rotations Over Regional Scale

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Abstract

Plant roots are often thought to selectively recruit their microbiome from the surrounding soil. But is this recruitment deterministic, or just an artifact of proximity? We put this question to a rigorous test using a Standardized Effect Size Recruitment Index (SES_RI), a permutation-based null model that strips away passive taxonomic overlap to isolate true host filtering. Applied to 340 paired bulk soil and root samples from 58 farms in the Northern Great Plains, sampled over three growing seasons (2021-2023): recruitment consistently and significantly outpaced neutral expectations, a signal robust enough to hold up against crop rotation as a potential confound. Fungi proved to be the dominant recruiters, assembling roughly 3.4-fold more deterministically than bacteria. This was independently confirmed by Sloan neutral community models showing over half of root fungal taxa exceeding neutral predictions. Soil health emerged as a strong predictor of fungal recruitment and crop nutrient composition. Moreover, it was the abiotic backbone of soil health, decoupled from diversity to avoid circular inference, that measurably tracked into fungal recruitment and crop nutrient composition. Exploring further, a feed-forward neural network trained on soil, weather, and management data explained 65% of held-out variance in fungal recruitment, turning an ecological pattern into a genuine forecast. Together, these findings frame microbiome recruitment not as a passive echo of the soil around a root, but as a predictable and learnable process governed by soil health. Potentially, this can be used as a lever for agricultural microbiome engineering.

O-11. Shift in Avian Metapneumovirus Epidemiology: Respiratory Disease due to Vaccine-Derived Strains in the U.S. Turkey Farms

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Abstract

Respiratory infections caused by emerging avian metapneumovirus (aMPV) subtypes A and B continue to cause significant economic losses in commercial U.S. turkey production despite vaccination programs. This study describes seven cases of respiratory disease detected on turkey farms in South Dakota (SD) and Minnesota (MN). The one-day-old poults were vaccinated with either aMPV-A or aMPV-B vaccine via coarse spray at the hatchery. The seven farms exhibited variable morbidity and mortality (7-50%). Dead birds had clear to mucoid secretions in the infraorbital and nasal sinuses, pneumonia, and fibrinous air sacculitis/peritonitis. Bacterial culture confirmed isolation of *Escherichia coli*, *Bordetella Avium*,

and *Ornithobacterium rhinotracheale* (ORT) from swabs collected from the lung and heart tissues. Salmonella was also detected in the cecum of four cases. PCR was positive for avian metapneumovirus (aMPV), and sequence analysis confirmed the relatedness of the detected viruses to vaccine strains. In conclusion, these findings indicate that severe respiratory disease and associated morbidity and mortality were caused by vaccine-related aMPV strains. This highlights a concerning shift in the epidemiology of circulating aMPV strains in U.S. poultry.

O-12. Which plant ferredoxins can support nitrogenase activity?

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Abstract

Biological nitrogen fixation is the process by which certain bacteria and archaea reduce atmospheric nitrogen to bioavailable ammonium using the enzyme nitrogenase. Engineering this pathway into non-nitrogen fixing-organisms, like plants, could reduce dependency on synthetic fertilizers. Nitrogenase activity requires delivery of reducing power by small electron-carrying proteins known as ferredoxins and flavodoxins, and successfully engineering nitrogen fixation into plants requires a mechanistic understanding of electron delivery through these proteins to the nitrogenase enzyme. Plants encode multiple ferredoxins that are localized to different organelles, raising an important question: *which plant ferredoxins can support nitrogenase activity*? To address this question, we expressed chloroplast-derived ferredoxins (Fd1) from plant species including *Oryza sativa*, *Arabidopsis thaliana*, and *Zea mays* in our model diazotroph, *Rhodopseudomonas palustris.* These ferredoxins were first expressed in a mutant background in which the heterologous ferredoxin was required to provide reducing power for both nitrogenase cofactor biosynthesis and catalysis. Under these conditions, only Fd1 from *O. sativa* supported some nitrogenase activity. We next investigated whether separating the electron-delivery requirements of nitrogenase cofactor biosynthesis and catalysis could improve nitrogenase activity supported by plant ferredoxins. To test this, we used a *R. palustris* strain containing a ferredoxin that can provide reducing power for nitrogenase cofactor biosynthesis. In this strain, Fd1 ferredoxin from *O. sativa*, *A. thaliana*, and *Z. mays* supported increased nitrogenase activity. Together, these results indicate that plant ferredoxins may not be able to support cofactor biosynthesis but can support catalysis. Providing a bacterial ferredoxin that supports cofactor biosynthesis increased nitrogenase activity with these plant ferredoxins. These findings provide new insights into the electron-delivery requirements of the nitrogenase pathway and establish a foundation for engineering biological nitrogen fixation in plants.

O-13. Microbial fermentation-driven extraction of plant proteins: A green alternative to chemical extraction

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Abstract

Growing demand for sustainable protein sources has increased interest in plant-based proteins from oilseeds, legumes and grains. However, conventional alkaline, salt, and enzyme-assisted extraction methods cause protein denaturation, require harsh chemicals, and incur environmental and economic costs. Emerging techniques such as deep-eutectic solvents, ultrasonication and microwave-assisted techniques face challenges in scalability and protein quality. This study evaluates microbial fermentation as a simple, biologically-driven

alternative for producing functional plant-proteins, using sunflower meal (SM) as model substrate. Filamentous-fungi produce diverse enzymes that facilitate protein release from lignocellulosic matrix while inducing beneficial protein modifications. We hypothesized that fermentation-assisted extraction would improve the overall properties and yield of sunflower proteins compared to conventional methods.

Pre-sterilized SM was fermented for upto 6 days using *Aureobasidium pullulans* and proteins (FEP) were extracted using neutral pH. Proteins extracted from unfermented SM at neutral (NEP) and alkaline pH (AEP) served as controls. Enzyme profiling showed significant production of cellulase, xylanase, amylase, pectinase, and protease, with peak activities after 2-3 days of fermentation. Fermentation for 24h gave the highest yield (64.7%), significantly higher than NEP (49.1%) but lower than AEP (70.8%). However, FEP exhibited better solubility and gelation properties than AEP, along with improved foaming and emulsification under acidic conditions (pH<5.0). These enhanced functional properties were associated with differences in the secondary and tertiary conformation, molecular weight, surface charges and reactive amino and sulfhydryl groups.

Overall, this study demonstrated that *Ap* fermentation enabled sustainable extraction of sunflower proteins with superior properties compared to chemical extraction for diverse food applications.

O-14. Enzymatic Conversion of Soybean Hulls Xylan to Prebiotic Oligosaccharides

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Abstract

Lignocellulosic waste management is an emerging global challenge, and one promising application is the production of prebiotic oligosaccharides through microbial and enzymatic conversion. Oligosaccharides are short-chain carbohydrates containing 2-6 sugar units that have gained immense interest as prebiotic ingredients due to their selective utilization by beneficial gut microorganisms. However, their production often relies on pure substrates and commercial enzymes, increasing production cost. Additionally, biomass-derived polysaccharides are structurally complex and are less efficiently hydrolyzed by purified enzymes. Therefore, there is a growing interest in developing effective and economical enzyme systems to produce oligosaccharides from complex agricultural biomass-derived polysaccharides. Filamentous fungi produce diverse carbohydrate-active enzymes in lignocellulosic substrates, making them promising candidates for developing economical crude enzyme systems.

We investigated crude xylanase production by *Aspergillus niger* (*An*) grown on soybean hulls (SH) and evaluated its potential for producing xylooligosaccharides (XOS) from SH-derived xylan. Crude xylanase was produced using *An* in SH-substrates through solid and submerged-state fermentation. Xylanase activity was quantified using the DNS assay. Hydrolysis was assessed at different enzyme loadings and reaction times. XOS and monosaccharides after hydrolysis were quantified by HPLC.

Crude enzyme produced by *An* demonstrated xylanolytic activity, reaching 22.04±2.24 U/mL endoxylanase activity on day 5 of solid-state fermentation. Crude enzyme dose of 6 U/mL produced the highest XOS concentration (78.79±14.20 mg/g substrate) within 10 min. Prolonged hydrolysis decreased XOS concentration while increasing xylose, reaching 35.5±0.7 mg/g substrate after 4 h. Overall, these findings demonstrate the feasibility of *An* crude enzymes for controlled XOS production from SH derived xylan.

O-15. *Azospirillum brasilense* Sp7 and cytidine as combination treatment on drought tolerance in peas

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Abstract

Sustainable strategies that integrate beneficial microbes and plant derived compounds may enhance stress tolerance. We hypothesized that *Azospirillum brasilense* Sp7, in combination with pea seedling exudates or cytidine, would enhance pea tolerance and recovery to drought stress.

Pea seedlings were germinated on water agar with or without exudates (1 mg/mL) and cytidine (0.1 M), then transplanted into sand-vermiculite medium with or without *A. brasilense* (10^4 CFU/mL). After four weeks in greenhouse conditions, drought was imposed for nine days. Shoot health was scored as percentage of healthy shoots daily based on wilting, shrinkage, and curling, while root dry weight was measured at harvest. Rhizosphere root microbiome composition was profiled using 16S rDNA amplicon sequencing.

In two replicate experiments, shoot health declined progressively under drought across all treatments, but plants receiving *A. brasilense* Sp7 with cytidine (ACyt) sustained shoot health 69.36% above *A. brasilense* Sp7 alone by day 9. A single replicate of a drought recovery study further showed that *Azospirillum* + cytidine treatments restored shoot health to ~95% compared with ~75% in the control after rewatering. Microbiome analysis showed that *A. brasilense* Sp7 inoculation under drought enriched Alphaproteobacteria while suppressing Gammaproteobacteria relative to uninoculated drought controls, a pattern that extended to the genus level, where *Rhizobium* and *Massilia* abundances mirrored this reciprocal trend. These findings indicate that combining *A. brasilense* Sp7 with cytidine improves pea drought tolerance and reshapes the rhizosphere microbial community in ways associated with enhanced plant performance.

O-16. Cross-scale multi-omics reveal concordant microbial responses and functional adaptations under salinity stress

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Abstract

Soil salinization is a global threat to agriculture, yet the extent to which salinity-induced microbial responses are consistent across environments and linked to plant-associated metabolic and functional changes remain unclear. To answer this question, we integrated a global meta-analysis spanning 52 studies across 14 countries and 6 continents with a controlled greenhouse experiment combining amplicon sequencing, untargeted root exudate profiling, and genome-resolved metagenomics. We found salinity to significantly drive both bacterial and fungal community composition, with several bacterial genera exhibiting concordant salinity responses across global and greenhouse scales. In the greenhouse, salinity significantly reshaped the root exudate profile, and multiple salinity-associated metabolites were associated with taxa that responded consistently across the two datasets. Genome-resolved metagenomics and effect size analysis showed a lower effect of salinity on functional potentials relative to taxonomic composition, highlighting a taxonomic-functional decoupling possibly due to functional redundancy. However, a small subset of functional potentials including nitrogen metabolism, respiration, and short-chain fatty acid metabolism was found to show markedly stronger responses to salinity. Finally, we constructed a multi-omics association network that revealed a classic example of a taxon-function-metabolite module that was responsive to salinity stress. Together, these results indicate that salinity

consistently restructures microbial communities across scales while selecting for a narrow range of salt-adapted taxa and specific functions through the plant root metabolome axis.

O-17. Nitrous Oxide Emissions in Single- and Two-Stage Partial Nitritation–Anammox Systems Under Hypersaline Conditions

Lin Gao

Abstract

Monitoring and controlling nitrous oxide (N_2O) emissions from partial nitritation–anammox (PN/A) processes has become increasingly important as this technology is more widely implemented. Here, N_2O emissions from completely mixed single-stage PN/A reactors, including fixed-bed and suspended-growth configurations, and two-stage PN/A systems were investigated for nitrogen removal under hypersaline conditions (4% salinity). Results clearly showed that, under hypersaline conditions, the single-stage PN/A reactors exhibited substantially lower N_2O emissions (approximately 1% of NH_4^+-N removed) than the two-stage PN/A systems, which exhibited emission factors of approximately 3% and 6% under high- and low-dissolved oxygen (DO) conditions, respectively, at bulk NH_4^+-N concentrations above 10 mg/L. Within the single-stage systems, N_2O emission factors ranged from 0.08% to 1.0% and from 0.09% to 1.5% of NH_4^+-N removed in fixed-bed and suspended-growth reactors, respectively. Bulk NH_4^+-N and NO_2^--N concentrations were identified as the main factors associated with N_2O emissions. Acetate addition reduced N_2O emissions by 52.5% and 72.4% in the fixed-bed and suspended-growth reactors, respectively. Quantitative PCR analysis showed increases in the denitrification-related functional genes *nirS*, *nirK*, and *nosZ* following acetate addition, whereas deammonification performance was not significantly affected. Ammonia-oxidizing bacteria and anammox bacteria detected in the PN/A reactor systems were primarily affiliated with the genera *Nitrosomonas* and *Candidatus Scalindua*, respectively. These results demonstrate the importance of reactor configuration, bulk nitrogen concentrations, and carbon availability in regulating N_2O emissions from PN/A systems under hypersaline conditions.

O-18. Enhancement and modeling of a dairy wastewater bioprocess: converting cheese whey into lactic acid and probiotic microbial biomass

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Abstract

In Mexico, cheese whey (CW) is considered dairy wastewater. This research investigated using CW and ultrafiltered cheese whey (UF-CW) as affordable substrates to cultivate *Lactobacillus acidophilus* LA-5 and *Lactococcus lactis* ATCC 11454. Fermentation was conducted for 36 hours at 30 and 37 ºC, with initial pH levels of 5 and 7. CW showed a higher capacity to support the growth of lactic acid bacteria. UF-CW was enhanced with yeast extract (YE) or corn steep liquor (CSL) and $CaCO_3$ to stabilize pH levels. Cell growth significantly improved; Lc. lactis achieved net increases of 1.97 and 1.57 Log_{10} CFU/mL with YE and CSL, respectively, while L. acidophilus showed a net increase of 1.35 Log_{10} CFU/mL with CSL. These results indicate that dairy wastewater can be repurposed as a low-cost cultivation medium for these bacteria. ASPEN simulation data reveal that lactose conversion and product concentration are key factors influencing the efficiency and cost-effectiveness of the lactic acid

production process. Future efforts should aim to improve fermentation yields and develop more efficient downstream recovery methods.

O-19. Constructing an Ordered *Campylobacter jejuni* Transposon Library

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Abstract

As a pathogenic bacterium that causes foodborne illnesses and is prevalent in developing countries, *Campylobacter jejuni* has a significant impact on agriculture, commercial food industries, and human health. Many non-essential genes impacting the pathogenicity of *C. jejuni* remain unidentified or understudied. We are currently refining a collection of *C. jejuni* mariner transposon mutants into a compact, ordered library for high-efficiency screening. Ordered transposon libraries readily enable identification of unknown bacterial genes required for survival, stress resistance, and host interaction. The 8,640 individual mutants previously generated were mapped and annotated relative to known regions in the genome. We identified 963 genes containing transposon insertions, representing around 58% of the *C. jejuni* genome, with an average of four insertions per gene. After removing strains with intergenic insertions or multiple transposons, the final library contains 905 unique, single-insertion mutants spanning 55% of all genes. To confirm each mutant gene, two representative mutants per gene were isolated, PCR-amplified using transposon and gene-specific primers and confirmed by gel electrophoresis. Verified mutants were stored in an ordered library that can be rapidly accessed and applied to screens investigating genes required for neutrophil infection, cold and milk survival, and nitric-oxide stress. These assays will identify genetic components that contribute to environmental persistence and pathogenicity, with implications for food safety and host colonization. The ordered library provides a resourceful tool for understanding *Campylobacter* biology and a broader discovery of factors that enable transmission and disease.

O-20. Active Site Mimicry Underlies Toxic Effector Neutralization in Human Gut Bacillota

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Abstract

Bacteria competing within the gut microbiome secrete toxin effectors and defend against intoxication with immunity proteins. Many known immunity proteins neutralize their cognate effector by sterically obstructing its active site. An immunity protein found in gut *Bacteroidales*, Tdi (T6SS DNase immunity protein), neutralizes the Tde nuclease effector (T6SS DNase effector) through a large structural rearrangement. We hypothesized that competitive antagonism in gut Bacillota uses Tde effectors, and immunity against Tde requires structural rearrangement. Tdi expression protected *Bacillus* species from Tde-mediated antagonism in competitive antagonism assays and *E. coli* from Tde-mediated toxicity. Crystal structures of three Bacillota Tde-Tdi complexes revealed a conserved neutralization mechanism, confirmed by interface mutagenesis in effector-immunity binding and bacterial competitive antagonism experiments. A polar interface is required for high-affinity binding, while a separate highly conserved Tdi motif ($P(\Phi)_4GG$) structurally mimics the toxin's own catalytic motif, displacing it from the active site and inactivating the effector. This structural mimicry was sufficient and necessary for complete inactivation of the toxin, as demonstrated with isolated $P(\Phi)_4GG$ motif

peptides and mutagenesis. The structural disruptions of Tde by Tdi increased its susceptibility to degradation in limited trypsin proteolysis experiments. Active site mimicry is a novel mechanism of effector inhibition that is conserved in polymorphic toxins from gut Bacteroidales and Bacillota.

O-21. More Than Meets the Eye: Uncharacterized Genes *yjbE* and *yciF* Shape *Escherichia coli* Colony Morphology

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Abstract

We recently showed that two uncharacterized genes, *yjbE* and *yciF*, contribute to *Escherichia coli* survival during high-dose ampicillin or ciprofloxacin exposure, with knockout of either gene reducing survival. Their functions remain unknown. Unexpectedly, the mutants exhibited changes in colony morphology. Time-lapse imaging on LB agar prepared without added NaCl (LBNa-) over 96 h showed that the *yjbE* mutant formed irregular, bubbled edges producing a cloud-like morphology, whereas the *yciF* mutant displayed a dense core and thin, irregular outer edges. These phenotypes were observed with and without antibiotic treatment, suggesting intrinsic gene-dependent processes rather than antibiotic-induced responses. Because *yjbE* is part of the *yjbEFGH* operon, which has been associated with osmotic stress responses and extracellular polysaccharide production, we examined the *yjbE* mutant across NaCl concentrations to test whether salt conditions influence its colony morphology. The cloud-like phenotype was most consistent at low and intermediate NaCl concentrations but disappeared at high concentrations. Similar changes occurred with KCl, suggesting sensitivity to osmotic or ionic conditions rather than a sodium-specific effect. Consistent with this, the *yjbE* mutant exhibited a reduced growth rate compared with wild type under elevated salt conditions. These findings reveal a previously unrecognized role for *yjbE* in *E. coli* colony architecture and suggest that environmental salt concentrations strongly influence *yjbE*-dependent morphology. Ongoing work will use M63 defined medium for tighter control of NaCl concentrations than LBNa-, along with time-lapse microscopy, promoter-reporter assays, and double-mutant analysis to further define *yjbE* function and determine whether *yciF* similarly responds to salt and osmolarity changes.

O-22. Building a Scalable *In Vitro* Platform to Assay Candidate ClpP Small-Molecule Activators

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Abstract

ClpP is a highly conserved bacterial protease whose activity is normally regulated by the ATP-dependent chaperone ClpX, which unfolds and delivers protein substrates into its proteolytic core. Small-molecule activators can bypass this regulation, allowing ClpP to degrade protein substrates without ClpX and ATP, leading to uncontrolled proteolysis and bacterial death. ADEP4 provides a well-established example of this mechanism and demonstrates the potential of ClpP activation as an antimicrobial strategy. We previously used computational

screening to identify AGI-6780 and Birabresib as putative ClpP activators and demonstrated through bacterial assays that both compounds inhibit target bacterial species. Computational screening and AI-assisted screening have since identified additional candidate ClpP activators, several of which are strongly effective against clinically relevant bacteria. Although molecular docking predicts these compounds bind to ClpP at known activator-binding sites, direct biochemical assays are needed to determine whether they activate ClpP as predicted. To establish this assay platform, we are purifying ClpP and ClpX and measuring casein degradation in the presence and absence of these compounds. We have purified *Bacillus subtilis* ClpP, confirmed by SDS-PAGE. A compound that hyperactivates ClpP should enable substrate degradation without ClpX and ATP, providing a direct biochemical readout of ClpP activation. Because candidate activators vary in antimicrobial activity across bacterial species, we are purifying ClpP and ClpX from multiple species. Comparing compound-dependent ClpP activation across species may reveal differences in antimicrobial activity arising from species-specific differences in ClpP itself. This scalable platform will enable biochemical testing and discovery of novel candidate ClpP activators.

O-23. Microbial Biofortification of DDGS with L-lysine via Co-culture Fermentation of *Corynebacterium glutamicum* and *Aureobasidium pullulans*

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Abstract

L-Lysine is an essential amino acid important for animal growth, protein synthesis, and immune function. Distillers dried grains with soluble (DDGS), a protein-and energy-rich co-product of corn ethanol industry, is widely used in animal feed but is limited by its relatively low lysine content. *Corynebacterium glutamicum* (*Cg*) is widely used for industrial L-lysine production, while *Aureobasidium pullulans (Ap)* produce extracellular enzymes that may improve substrate utilization during fermentation. Their co-culture may therefore provide a complementary approach to simultaneously modify the DDGS matrix and promote L-lysine enrichment. This study evaluated solid-state fermentation (SSF) of DDGS using *Cg* and *Ap*, individually and in co-culture, to improve nutritional properties with particular emphasis on L-lysine biofortification. DDGS was hydrated, pH-adjusted, autoclaved and inoculated with either *Cg, Ap* or *Ap+Cg*; an uninoculated DDGS served as the control. SSF treatments were conducted in triplicate at 30˚C for five days. The *Cg+Ap* co-culture showed the greatest increase in crude protein from 32.8% to 36.4%, corresponding to an 11.1% increase, while the control remained relatively unchanged. In a preliminary submerged-fermentation experiment, *Cg* increased L-lysine content of DDGS from 0.76% to 0.92%(w/w), representing a 19.7% increase.

These findings demonstrate the potential of microbial fermentation to improve overall nutritional attributes of DDGS. The increase in total lysine following *Cg* fermentation supports L-lysine biofortification, while the greater increase in crude protein with the *Cg+Ap* co-culture suggests complementary effects of two microorganisms. Ongoing lysine analysis of the SSF treatments will determine whether co-culturing *Cg* with *Ap* provides additional benefits for L-lysine enrichment.

O-24. Nanogels for Oncological Drug Delivery Synthesized from Thermophile-Derived Biopolymers

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Abstract

Exopolysaccharides (EPS) derived from a thermophilic bacterial strain were conjugated with naturally occurring casein micelles to form biobased core-shell structures. Using curcumin as a model hydrophobic drug, the resulting nanoparticles exhibited consistent morphology, size, and structural integrity. SEM and TEM imaging confirmed successful core-shell assembly with distinct hydrophobic and hydrophilic domains, consistent with amphiphilic self-organization. Dynamic light scattering revealed an average hydrodynamic diameter of ~240 nm with an acceptable polydispersity index, supported by suspension stability under cool, inert conditions. Encapsulation efficiency, loading capacity, and release kinetics demonstrated anomalous diffusion behavior, suggesting variable swelling within the polymeric matrix. Current work incorporates redox-labile cystamine linkages to couple the EPS shell to the casein core, enabling environmentally responsive disassembly. This platform shows strong potential for targeted cancer therapy, with future development focused on PEGylation and folate-ligand decoration for enhanced circulation and tumor specificity.

O-25. Bacterial Antagonism Limits the Growth of Spore-Forming *Caryophanales* in the Rhizosphere

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Abstract

Bacillus sensu lato (BSL) are fast-growing, endospore-forming bacteria of the *Caryophanales* that are commonly found in soil. Even though they grow rapidly, they usually make up less than 1% of the soil bacterial community and are even less in the nutrient-rich rhizosphere. Upon introduction of synthetic root exudates to bulk soil, BSL numbers did not increase. We hypothesized that other soil bacteria suppress BSL populations. Using *Bacillus pseudomycoides* as a test strain, we found 124 isolates that inhibiting its growth. 16S rRNA gene sequencing revealed 3 *Alphaproteobacteria*, 6 *Betaproteobacteria*, 6 *Gammaproteobacteria*, 3 *Streptomyces*, and 20 *Caryophanales* species. Most of these antagonistic isolates also inhibited four other BSL. More than 70% of the BSL isolates we tested were also able to inhibit other BSL strains. When we tested multiple isolates in artificial soil microcosms, BSL growth was limited, and a large proportion of the cells formed endospores in the presence of antagonists. This suggests that interactions with other bacteria may limit BSL populations by either inducing sporulation or suppressing germination. *BSL* population did not increase when grown in sterile soybean soil with antagonists, non-antagonists, or soil bacterial extracts, but increased when grown alone. These results support the idea that other soil bacteria can limit BSL populations even when fresh nutrients are available. Our current work focuses on whether BSL form spores, whether those spores can germinate, and whether the germinated cells are able to outgrow as normal vegetative growth. We are also comparing the proportion of spores in different soil types.

O-26. Comparative Effects of *Azospirillum brasilense* on Hydroponically Grown Lettuce Using Tap and Sugar Beet Processing Water

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Abstract

Alternative water sources may provide opportunities for controlled-environment agriculture, but differences in microbial communities may influence their use. This study evaluated whether *Azospirillum brasilense*, a plant growth-promoting bacterium, influences hydroponic

microbial communities differently when lettuce is grown using tap water (TW) or sugar beet processing wastewater (WW). Lettuce seedlings were inoculated with or without *A. brasilense* during establishment and subsequently grown hydroponically for four weeks. Water chemistry, culturable bacterial populations, and bacterial community composition were evaluated.
In TW, *A. brasilense* had limited effects on measured parameters. In WW, inoculation was associated with significantly lower total dissolved solids ($p=0.025$) and lower bacterial counts on EMB agar ($p=0.032$). No colonies displaying the characteristic metallic-green sheen associated with *Escherichia coli* were observed. 16S rRNA sequencing showed that WW generally supported greater bacterial richness and diversity than TW at Week 4. Bray-Curtis analysis showed separation of bacterial communities primarily by water source, with less separation associated with *A. brasilense* within each water source. Pseudomonadota dominated Week-4 communities, while the relative abundances of other major phyla varied by water source and treatment.
Overall, water source was a major factor associated with bacterial community structure, while microbial responses associated with early *A. brasilense* inoculation varied depending on water source.

O-27. Proximity-ligation metagenomic sequence analysis reveals novel ammonia oxidizing bacteria growing in full-scale municipal wastewater treatment bioreactors

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Abstract

Nitrification is an important step during the biological wastewater treatment of municipal wastewater to satisfy permit requirements, avoid ammonia toxicity, and prevent eutrophication. This biochemical process is carried out by ammonia-oxidizing microorganisms (AOM). Our current understanding of AOM comes from a relatively small number of laboratory isolates of *Nitrosomonas* spp. that may not adequately represent the AOM in genuine wastewater bioreactors. Samples were collected from 10 municipal wastewater facilities across Minnesota spanning diverse treatment designs (conventional activated sludge, membrane bioreactor, sequencing batch reactors, and anaerobic/oxic systems). Samples were analyzed by proximity-ligation metagenomic sequence analysis, enabling the construction of community profiles of 50-100 metagenome-assembled genomes (MAGs) per sample. Within these MAGs, numerous *Nitrosomonas* spp. were identified that primarily clustered phylogenetically with each other rather than the genome sequences of previously isolated Nitrosomonas spp. Similarly, the sequences of the ammonia monooxygenase (amo) operons in these MAGs were similar to each other but divergent from previously published genomes. Because most previous research on nitrifying bacteria in wastewater treatment bioreactors has involved PCR, an in silico analysis of four commonly used PCR primer sets targeting amoA primers was also performed. Each of these primer sets likely would have failed to amplify all of the *amoA* genes in each sample. Our research demonstrates, therefore, that proximity-ligation metagenomic sequence analysis reveals novel, phylogenetically distinct *Nitrosomonas* populations in wastewater bioreactors, some of which would not have been detected by previously developed PCR based approaches.

O-28. Biofilms as Biofoundries: Unlocking the Hidden Microbiome of Wastewater Treatment Biofilms

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Abstract

Microorganisms in wastewater reclamation facilities form complex biofilms essential for organic degradation and nutrient recovery, yet much of their uncultivated functional diversity remains unexplored. We hypothesized that environmental wastewater biofilms across different treatment stages harbor distinct, functionally specialized microbial consortia whose genomic diversity encodes pathways for carbon sequestration, nutrient transformation, and xenobiotic degradation. Using long-read sequencing across four distinct niches (activated sludge-S1AcSg, rusted pipe-S2RstP, concrete wall-S3BioCw, and biofilm-S4BioSwS), we reconstructed metagenomes to perform high-resolution taxonomic profiling, full-length 16S rRNA analysis, and metabolic pathway modeling. The analysis recovered 44 high-quality metagenome-assembled genomes (MAGs) spanning diverse bacterial phyla. A shared metabolic core supported acetoclastic methanogenesis, alcohol biosynthesis, and nitrogen cycling across samples, while the S4BioSwS emerged as the most phylogenetically and functionally diverse. S4BioSwS uniquely encoded pathways for thiosulfate oxidation, xenobiotic degradation (atrazine, dioxins, caprolactam), and enriched carbonic anhydrase (CA) potential (32 of 81 total CA genes). The key novelty lies in full-length 16S rRNA analysis, which uncovered 82 out of 111 contigs representing potential novel bacterial taxa, with lineage novelty reaching up to 83.3% in specific niches. The niches could be characterized by indicator species such as *Acidothermus cellulolyticus* (acidic), *Halomonas* ssp. (saline), *Deinococcus radiophilus* (radiation), and *Sterolibacterium denitrificans* (anoxic) from the respective 4 samples**.** In conclusion, rapid city wastewater biofilms harbor distinct, resilient functional networks and uncharacterized biocatalysts capable of driving scalable bioprocesses. This study has significant potential to impact the circular bioeconomy by establishing a high-resolution genomic framework for waste-to-resource conversion, biological decarbonization, and sustainable bioremediation.

O-29. Cross-Species Circulation and Genomic Architecture of H5N1 Clade 2.3.4.4b in Dairy Cattle and Domestic Cats in South Dakota

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Abstract

Background: Highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b emerged in U.S. dairy cattle and domestic cats in early 2024. The Animal Disease Research and Diagnostic Laboratory received cases related to H5N1 outbreaks in dairy cattle and domestic cats in South Dakota. We investigated whether infection in dairy cattle and domestic cats represented shared transmission chains or independent spillover events.
Methods: Milk samples from dairy cattle were submitted to ADRDL in May and June 2024 to test for HPAI by realtime RT-PCR and virus isolation. Brain samples were collected from domestic cats for PCR and virus isolation. Positive samples were further processed for whole-genome sequencing. Assembled whole genomes were further analyzed for phylogenetics, molecular clock dating, minor variant calling, and structural modeling. Defective viral genomes and minor WT variant populations were mapped and quantified using high-depth read alignments.
Results: Three virus isolates from cattle samples were sequenced and two from domestic cats. The cattle viruses belonged to genotype B3.13, forming a local dairy cluster dating to March 2024. In contrast, the two feline viruses belonged to genotype D1.1 and grouped with distinct regional wild bird and mammalian lineages, ruling out direct cow-to-cat transmission.

Both domestic cat sequences gave close match with sequences reported from MN and KS. Bovine PB2 segments exhibited marked structural heterogeneity, dominated by 5-prime micro-deletions (10 bp and 15 bp; 76.9% to 95.2% frequency) and large internal deletions (DI-1: 1,680 bp; DI-2: 1,985 bp). Full-length intact minor wild type (WT) variants persisted at 4.8% to 23.1% frequency, maintaining several hundred-fold coverage across the catalytic core to serve as replication-competent genomes. In domestic cats, one fatal case acquired consensus PB2 D701N, which structurally alters the nuclear localization signal to enhance mammalian importin binding. Hemagglutinin receptor-binding markers retained avian-like alpha-2,3 sialic acid specificity across all isolates.
Conclusions: The dairy cattle B3.13 and domesic cats D1.1 H5N1 cases in South Dakota represent independent introductions. The coexistence of defective interfering RNAs with persistent minor WT variants in dairy cattle highlights complex viral population dynamics that require deletion-aware genomic surveillance.

O-30. deepFLUpred: A Deep Learning-Based Framework for Genomic Characterization, Subtyping, and Pathogenicity Prediction of Avian Influenza Viruses

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Abstract

Rapid subtyping and pathogenicity assessment of avian influenza viruses are essential for genomic surveillance and outbreak response. Conventional approaches rely on sequence alignment or BLAST searches against curated reference databases, which are computationally intensive and sensitive to reference selection. We present deepFLUpred, a deep learning framework that classifies the hemagglutinin (HA) and neuraminidase (NA) gene segments and predicts pathogenicity directly from nucleotide sequence, without alignment. HA and NA nucleotide sequences retrieved from GISAID were deduplicated using CD-HIT across a range of sequence identity thresholds. Training and test partitions, including all cross-validation folds, were constructed so that no sequence cluster was split across partitions, eliminating leakage from near-identical submissions. Each sequence is tokenized with the DNABERT-2 tokenizer, encoded by a pretrained transformer to obtain contextualized nucleotide embeddings, and classified by a bidirectional long short-term memory (BiLSTM) network. A dedicated module localizes the HA1/HA2 cleavage site through open-reading-frame translation and motif search, then predicts pathogenicity, highly pathogenic avian influenza or low pathogenic avian influenza, from cleavage-site basicity and a BiLSTM classifier trained on the flanking nucleotide window, evaluated independently for chicken- and duck-derived sequences. Held-out test accuracy exceeded 98 percent, with Matthews correlation coefficient above 0.98, for both HA, 16 subtypes, and NA, 9 subtypes, subtyping at 99 percent sequence identity, and exceeded 99 percent for pathogenicity classification across both host groups. By operating directly on raw nucleotide sequences without reference alignment, this approach offers a scalable, reference-independent alternative to conventional BLAST-based pipelines for rapid, real-time genomic surveillance as avian influenza sequence data continue to accumulate worldwide.

O-31. Evidence for Epigenetic Adaptation Enabling *Escherichia coli* to Survive Low-Dose Antibiotic Exposure Across Generations

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Abstract

Antibiotic resistance is a global health threat. Exposure to antibiotic concentrations below the minimum inhibitory concentration (MIC) can promote resistance emergence. We investigated the response of *Escherichia coli* to low doses of antibiotics using apramycin (Apr). Although the MIC is commonly treated as fixed, Apr MIC was population-density dependent, increasing with cell density before plateauing. On solid media containing sub-MIC Apr, most cells were killed, but rare survivors formed colonies initiated, on average, by single cells. Whole-genome sequencing identified no resistance-conferring mutations, and survivors regained susceptibility after passage without Apr, demonstrating a reversible, non-genetic phenotype. Apr activity remained stable for over 72 hours, excluding antibiotic degradation as the primary cause of survival. Apr-exposed populations displayed reduced lag times and enhanced growth upon re-exposure, demonstrating transient cellular memory. Each surviving cell expanded to millions of descendants during Apr exposure, demonstrating that the survival phenotype persisted across generations. Phenotypic reversibility, the lack of resistance conferring mutations, and cellular memory upon re-exposure are most consistent with epigenetic adaptation, through which heritable changes in cellular state arise and persist across generations without modification of the DNA sequence. RNA sequencing identified 255 differentially expressed genes in Apr-treated colonies, with network analysis highlighting candidate pathways underlying survival. Targeted knockout mutants are being tested for altered Apr survival, while promoter-reporter constructs are being developed to determine whether expression states exhibit transient mother-to-daughter inheritance. These findings support epigenetic adaptation as a mechanism enabling *E. coli* to survive low-dose antibiotics across generations and identify candidate pathways underlying this adaptive state.

O-32. Genetic determinants of beta-lactam resistance in the gut microbe *Eggerthella lenta*

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Abstract

Many members of the human gut microbiota are capable of causing infection outside the gut. For example, the Actinobacterium *Eggerthella lenta* occasionally causes both local and systemic infections. Notably, it has a large pangenome and extensive strain variation. Due to limited literature on *E. lenta's antimicrobial* sensitivity profile, infections are treated with various antibiotics, including broad spectrum penicillin-based antibiotics with βlactamase inhibitors like piperacillin-tazobactam. There is relatively little evidence to support the use of these drugs in *E. lenta* infections. We aimed to quantify β-lactam resistance in *E. lenta* and to identify genes linked with resistance. We performed minimum inhibitory concentration (MIC) assays for penicillin G and amoxicillin trihydrate on a collection of *E. lenta* isolates (n= 32) and observed most strains were resistant (96%), though the degree of resistance varied (MIC range 1-3 and 4-16 µg/mL for penicillin G and amoxicillin respectively). We analyzed coding sequences from 284 *E. lenta* genomes using eggNOG database for β-lactamase, Penicillin Binding Protein (PBP), and transpeptidase gene families. Despite identifying 9 putative β-lactamase genes, no correlation between any β-lactamase genes and MIC existed, and we confirmed there was no beta-lactamase activity with nitrocefin assay. However, MIC was associated with sequence variation in a conserved PBP gene family. We are currently using genome-wide association analyses to identify alternative genes that could be responsible for variation in β-lactam resistance. Our findings will inform the treatment regimen for *E. lenta* infections and shine some light on the mechanisms of antibiotic resistance in the human gut microbiota.

O-33. Elucidating regulatory elements involved in cell death and cell survival in pyroptosis.

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Abstract

Background: Pyroptosis, an inflammatory-regulated cell death, is triggered by pathogen-associated molecular patterns (PAMPs). PAMPs activate inflammasomes, leading to the activation of inflammatory caspases, which cleave gasdermin-D (GSDMD), generating an N-terminal GSDMD fragment that oligomerizes and forms membrane pores. However, GSDMD pore formation does not always result in cell death, as the endosomal sorting complex required for transport (ESCRT) can repair the membrane by removing GSDMD-containing membrane sections. The upstream regulators determining whether the membranes are repaired or not remain unclear.
Methods: We employed flow cytometry to detect and quantify membrane damage using propidium iodide uptake in non-fixed cells. We sorted cells with high and low surface GSDMD levels and performed proteomics analysis on these populations to identify regulatory factors. We used immunoprecipitation to identify ESCRT proteins interacting with Caspase 2. We applied shRNA gene silencing by lentiviral particles to knock down these components, assessing their role in membrane repair upon pyroptosis activation.
Results: Our results show variability in pyroptosis susceptibility within human cell subpopulations of the same cell type, with some cells surviving the stimulus. Our preliminary data suggests that Caspase-2, activated by PAMPs in pyroptosis, may negatively regulate membrane repair. We identified ALIX as a key player in cell survival via membrane remodeling during pyroptosis. Disruption of ALIX correlated with increased cell death following extensive membrane damage.
Implications: These findings enhance our understanding of pyroptosis sensitivity correlating with the membrane self-healing potential of cells in infection and inflammation, potentially paving the way for novel drug development strategies.

O-34. A major CD4 Helper T cell subset induced during Malaria provides suboptimal B cell help and is promoted by adenosine signaling

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Abstract

Plasmodium, the parasite that causes malaria, infects over 250 million people and leads to nearly 600,000 deaths yearly worldwide. Robust antibody responses are essential for controlling the infection. T follicular helper (T_{FH}) cells play a pivotal role in supporting the production of B cell activation and antibody secretion by B cells. Clinical and experimental studies show that a distinct subtype of helper T cell emerges during *Plasmodium* infection, termed “T follicular helper type 1 (T_{FH1}) cells. T_{FH1} cells secrete IFN-□, a cytokine that has been known to suppress humoral responses, and these cells have been identified in viral and bacterial infections. However, their role in modulating humoral responses remains controversial. Given the varied impact of T_{FH1} cells on host immunity, we investigated their functional influence as well as mechanisms involved in their formation during *Plasmodium* infection. We identified that independent of their IFN-□ secretion, T_{FH1} cells induce suboptimal B cell responses and provide less parasite control compared to standard T_{FH} cells. We also identified that T_{FH1} cells exhibit elevated expression of the IL-12 and adenosine receptors. Il-12 is an inflammatory cytokine and chemical messenger. During malaria, increased cell death and inflammation triggers increased extracellular ATP that gets converted to immunosuppressive adenosine. Manipulation of these pathways demonstrated that both IL-

12 and adenosine signaling promote T_{FH1} formation. Ongoing studies are investigating potential mechanisms impairing the ability of T_{FH1} cells to help B cells. These results identify critical pathways that could potentially be targeted to modulate immunity to malaria.

O-35. Characterizing Ebolavirus Localiza on and Neuroinflamma on in the Murine Central Nervous System (CNS)

Omar Aris Zabal, Blake Bernauer, Jack Siemering, Hanora Van Ert, Paige Richards, Bernd Zinselmeyer, Andrea Marzi, Wendy Maury, Sabrina Tan

Abstract

Ebola virus (EBOV) infec on is rare yet lethal. Infec on is characterized by hemorrhagic fever, systemic hyperinflamma on, and mul organ failure in part due to cytokine storm. While the central nervous system (CNS) is not considered a primary site of early infec on, some survivors suffer neurological sequelae with the risk of relapse. How Ebola enters the CNS and its cellular targets remains unclear.

Interferon-α/β receptor knockout (IFNAR-/-) mice were infected with wild-type EBOV to recapitulate key aspects of disease pathogenesis. Infec on was performed under biosafety level-4 (BSL-4) condi ons at Rocky Mountain Laboratories. Mice were euthanized at 5 days postinfec on (DPI). RT-qPCR was used to quan fy viral RNA and cytokine transcripts, while mul plex IHC and RNAScope immunofluorescence analysis supported single-cell mapping of infected and ac vated brain cell popula ons.

EBOV viral proteins localized predominantly with microglia and macrophages. Neurons, astrocytes and endothelial cells exhibited minimal direct infec on, although astrocytes were frequently adjacent to infected cells. Infected brains exhibited reduced signals in neurons, endothelial cells, and ght junc on proteins. Inflammatory cytokines IFN-α, IFN-β, CXCL10, ISG15, and GBP5, were upregulated in the CNS by microglia, macrophage, astrocytes, and endothelial cells, consistent with a strong an viral interferon-s mulated gene response within the CNS.

These results support a ‘Trojan horse’ model in which infiltra ng macrophages carry EBOV into the CNS, seeding infec on of resident microglia and triggering a local type I interferon–driven an viral proinflammatory response. This work highlights innate immune pathways in the brain as poten al targets to mi gate long-term neurological complica ons of Ebola virus disease.

O-36. Investigation of potential nitrogenase-like genes in a human gut bacterium, *Raoultibacter spp.*

Angshula Khan and Cecilia Noecker

Abstract

Gut microbes play essential roles in host metabolism and immune function. They acquire nitrogen, an essential element, from various sources, which are thought to include largely host-digested proteins. We analyzed nitrogen metabolism in the widespread bacterial family *Eggerthellaceae* and made a surprising observation of genes linked to nitrogen fixation (known as the *nif* pathway genes) in genomes from genus *Raoultibacter*. *Raoultibacter nif* genes have sequential and structural similarity with those from known nitrogen fixers, including all cofactor biosynthesis genes and a conserved active site in the nitrogenase *nifH*. We are investigating whether *Raoultibacter* uses these genes for nitrogen fixation and/or other metabolic functions. Quantitative PCR showed expression of *nifH* during growth in complex media. Interestingly, *Raoultibacter* growth was severely inhibited (72.87%) by the addition of tungstate, which inhibits nitrogenase and other molybdopterin-dependent enzymes. We plan to further quantify nitrogenase expression and activity and other metabolic changes across different

environmental conditions. This study will shed new light on the flow of nitrogen in the gut ecosystem and the functions of unknown enzymes in the human gut.

O-37. A nitrogenase-like enzyme involved in sulfonate assimilation

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Abstract

Sulfonates are abundant organosulfur compounds that play key roles in microbial sulfur cycling, yet their degradation pathways have been predominantly characterized under oxic conditions. In contrast, the mechanisms underlying anaerobic sulfonate catabolism remain poorly understood. Recent studies identified a nitrogenase-like enzyme called isethionate reductase (Isr), which is required for isethionate assimilation under anoxic conditions in the purple nonsulfur bacterium, *Rhodobacter capsulatus*. However, we discovered that Isr in another purple nonsulfur bacterium, *Rhodopseudomonas palustris*, is required to assimilate taurine, isethionate, and the longer-chain sulfonate, hexanesulfonate. Using random-barcoded transposon sequencing (RB-TnSeq) and growth assays with strains lacking Isr, we found Isr is required for utilization of taurine, isethionate, and hexanesulfonate under anoxic conditions but is dispensable under oxic conditions. Metabolite profiling showed indicates that isethionate accumulates during growth on taurine, suggesting a novel metabolic route in *R. palustris* that connects anaerobic taurine utilization to Isr-dependent C-S bond cleavage. We also provide evidence that sulfite (SO_3^{2-}) is generated during growth with sulfonates as a sulfur source, providing insight into the mechanism of Isr in reducing sulfonates. Together, these findings expand the known substrate scope and physiological role of nitrogenase like enzymes.

O-38. A divergent 2-oxoacid:ferredoxin oxidoreductase links propionate metabolism and isoleucine biosynthesis in *Rhodopseudomonas palustris*

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Abstract

A family of reversible, ferredoxin-dependent enzymes, the 2-oxoacid:ferredoxin oxidoreductases (OFORs), mediate a variety of (de-)carboxylation reactions in central carbon metabolism and amino acid biosynthesis and degradation. However, determining OFOR function from sequence annotations is challenging. Thus, by improving our understanding of the reactions catalyzed by OFORs *in vivo*, we might be able to improve functional annotations and engineer OFORs into relevant pathways for producing amino acids or other high-value bioproducts.

To explore OFOR function *in vivo* we selected *Rhodopseudomonas palustris,* a geneticallytractable anoxygenic phototroph, as a model system. *R. palustris* can grow on a wide range of carbon sources including citraconate (an intermediate of L-isoleucine biosynthesis) and Lleucine and encodes multiple OFORs of unknown function. Therefore, we hypothesized that one or more of these unexplored OFORs might facilitate growth on those substrates.

By combining transposon mutagenesis, in-frame gene deletions, and bioinformatic investigations of *R. palustris*' OFOR homologs, we were able to identify a single OFOR (RPA3195) that is responsible for growth on both citraconate and L-leucine. We also show that RPA3195 diverges at the sequence level from all biochemically/structurally characterized OFORs—highlighting its unique role in cellular metabolism. Additionally, we demonstrate that

this dual functionality is not fully conserved among the rest of the clade of Proteobacterial OFORs sharing homology to RPA3195.
Our work establishes that a single OFOR can catalyze two physiologically distinct reactions *in vivo* and lays the foundation for future research into the biochemical basis of substrate specificity in this family of industrially relevant enzymes.

O-39. Coordinated Control of Iron Acquisition, Utilization, and Oxidative Stress Defense via sRNA–Fur Network in *Campylobacter jejuni*

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Abstract

Iron is a critical micronutrient for *Campylobacter jejuni*, a major cause of bacterial foodborne disease and an etiologic agent of sheep abortion in the United States. *Campylobacter* must scavenge this limited resource in the intestinal tract while avoiding toxicity, a process classically controlled by the transcriptional regulator Fur. However, substantial iron-responsive gene expression has been shown to occur in *Campylobacter* independent of direct Fur binding, pointing to additional post-transcriptional layers of control. We previously showed that the conserved small RNAs (sRNAs) CjNC110 and CjNC140 are essential for chicken colonization and that their deletion alters expression of iron-regulon transcripts. In this study we tested the hypothesis that these sRNAs act as a checks-and-balances system in coordination with Fur to maintain iron homeostasis. Phenotypic assays demonstrated that both sRNA mutants are severely impaired for growth under iron-replete and iron-deplete conditions, in contrast to normal growth of the Δfur mutant. Multi-omics analyses (RNA-seq, MS2-affinity purification coupled to RNA-seq, proteomics, and qPCR) demonstrated that CjNC110 primarily represses iron-acquisition systems under iron excess while CjNC140 exhibits RyhB-like iron-sparing behavior. EMSAs and phenotypic assays demonstrated that both sRNAs bind critical mRNAs and alter sensitivity to oxidative stress, with CjNC110 acting as a positive regulator and CjNC140 acting as a negative regulator of the oxidative-stress response. Together these data indicate that CjNC110 and CjNC140 operate in concert with Fur to provide post-transcriptional control of iron acquisition, storage, utilization, and oxidative-stress defense, thereby enabling precise adaptation to fluctuating iron availability and supporting virulence of *C. jejuni* IA3902.

O-40. Safety and protective efficacy of a novel U.S. strain–derived live attenuated avian metapneumovirus subtype B vaccine in chickens

Mohamed Selim, Kada Parfait, Noreen Bashir, Ahmed Mansour, Ahmed Abdalla, Ravi Gupta, Tamer A. Sharafeldin, Sunil Mor

Abstract

Avian metapneumovirus subtype B (aMPV-B) emerged in U.S. poultry in early 2024. The aMPV-B/chicken/USA/SD-24/P6 strain was isolated from a chicken flock affected by swollen head syndrome (SHS) using primary turkey tracheal cells. Following successful adaptation to Vero E6 cells, the isolate was serially passaged to generate three live-attenuated vaccine candidates at different passage levels: vaccine candidate 1 (V1), vaccine candidate 2 (V2), and vaccine candidate 3 (V3).
The safety and efficacy of the three candidates were evaluated in three in vivo experiments: loss of virulence, protective efficacy following vaccination and challenge, and attenuation stability. In the first experiment, one-day-old specific-pathogen-free (SPF) chickens were

inoculated with V1, V2, or V3 via the nasal–ocular route, alongside negative- and positive-control groups. Birds were monitored for clinical signs, choanal cleft virus shedding, and gross and histologic lesions at 3, 5, 7, and 10 days post-inoculation (DPI).
In the second experiment, four groups of one-day-old SPF chickens (n = 25 per group) were vaccinated via the nasal–ocular route with V1, V2, or V3 at one day old, while the fourth group remained unvaccinated as a control. Vaccinated and control birds were subsequently challenged with a virulent U.S. aMPV-B strain at 18 days of age (18 days post-vaccination) to evaluate protective efficacy. In the third experiment, the candidate demonstrating the most significant safety and efficacy profile was subjected to six consecutive bird-to-bird passages to assess attenuation stability.
Among the candidates evaluated, V2 demonstrated the most favorable profile. It was associated with significantly lower clinical-sign scores and gross and histologic lesion scores while retaining sufficient replication in the respiratory tract. V2 also induced ELISA antibody responses with neutralizing activity against both U.S. aMPV subtypes A and B. Following challenge with the virulent U.S. aMPV-B strain, V2 provided significant protection and was associated with complete virus clearance throughout the 7-day post-challenge observation period. In addition, V2 maintained a stable attenuation, with no evidence of reversion to virulence after six serial bird-to-bird passages.
Because no live attenuated aMPV-B vaccines derived from U.S. field strains are currently available, V2 represents a promising vaccine candidate that could strengthen existing aMPV control strategies and help reduce production losses associated with aMPV infection in the U.S. poultry industry.

O-41. Metagenomic Functional Signatures for Real-Time Predictive Monitoring of Cyanobacterial Bloom Assessment and Biofilm Marker Discovery

Connor Johansen-Sallee

Abstract

Harmful algal blooms (HABs), driven by cyanobacteria such as Microcystis, pose a critical threat to global freshwater security. Standard environmental monitoring often provides reactive data, underestimating the true ecological state after cyanobacteria draw down nutrient levels, failing to capture the risk of biofilm establishment within water infrastructure. To develop a proactive monitoring framework, we conducted a two-year longitudinal study (2024–2025) of Lake Mitchell, South Dakota, integrating environmental chemistry with advanced computational metagenomics.
We used 16S/18S rRNA amplicon sequencing (QIIME2) to profile microbial community structure and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) to predict functional enzyme repertoires. Temporal analysis revealed significant ecological trajectory toward eutrophic conditions in 2025, characterized by increased alkalinity (pH 8.58 to 8.79) and dissolved nutrient depletion. Crucially, community shifts showed an increase in the HAB-former Microcystis alongside the heterotrophic degrader Flavobacterium. Functional inference reinforced this finding, revealing enrichment of metabolic pathways linked to stress and replication, which precedes settlement and biofilm initiation: specifically, elevated ATP-dependent helicase (EC:3.6.4.12) and NADH dehydrogenase (EC:1.6.5.3). These enzyme signatures reflect an intensified state of oxidative stress handling and metabolic turnover, characteristic of blooms with high surface-attachment potential. These findings show predictive functional metagenomic signatures serve as strong, sensitive markers of bloom development and the potential for harmful bacteria to colonize water treatment infrastructure. These signatures operate independently of short-term nutrient fluctuations and offer insights into conditions that may lead to surface-associated microbial growth—which is critical for protecting municipal water supplies and public health from HAB contamination.

Poster Abstracts

P-01. Improving Mass Transfer in Methanotrophic Bioreactors using Nanobubble Technology

Daniel Ocampo[1], Ella Laverman[1], Keenan McQuistion[1], Emma Milbrodt[1], Samantha Harris[1], Dipayan Samanta[1], Rajesh K. Sani[1,2,3]

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Abstract

Methanotrophic products are of interest to the biomedical industry due to their environmental stability and variety of utilities. This project focuses on designing a reactor for more efficient methane processing using methanotrophs and nanobubble technology for biomedical applications. The C-H bond in methane is relatively unreactive and requires high activation energy in traditional chemical reactors, thus by using methanotrophs in a bioreactor, the process is less costly and more efficient. However, slow growth rates in previous methane-processing bioreactors hinders large-scale bioprocessing. Using nanobubble technology assists in distributing methane to methanotrophs, increasing large-scale bioprocessing growth rates, due to their sustained stability in media compared to larger bubbles. This stability is created from nanobubble size and stronger internal pressure due to their high surface area to volume ratio and negative surface charge. Research shows nanobubbles increase the volumetric mass transfer coefficient; however, further research is needed to understand how to more effectively utilize nanobubbles in a larger-scale bioreactor.

P-02. *Myxococcus xanthus* globins exhibit heme binding, providing insight into proteins that enhance *Gluconobacter oxydans* growth

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Abstract

Myxococcus xanthus is an obligately aerobic soil bacterium that encodes two predicted globins, heme-binding proteins that may contribute to oxygen utilization and cellular stress responses. These globins may serve distinct functions: HbO globins can facilitate oxygen utilization, whereas HbN globins are associated with nitric oxide detoxification. Previous work in the Bretl Lab found that expression of the *M. xanthus* globin HbO improves growth of the industrially relevant bacterium *Gluconobacter oxydans*, while expression of HbN does not. However, the mechanism underlying these differing effects on *G. oxydans* growth remains unclear. This project characterized HbO and HbN to assess heme binding and tested the predicted roles of specific histidine residues in their heme-binding pockets. Recombinant wild-type and histidine-to-alanine globin variants were purified and analyzed by UV spectrophotometry to assess heme-associated absorbance spectra. Both wild-type proteins exhibited absorbance spectra consistent with heme binding. Mutation of the predicted heme-coordinating histidine residues eliminated heme-associated absorbance in both proteins, confirming their predicted roles in heme coordination within the pocket. These results establish that HbO and HbN bind heme and provide a foundation for determining how their heme-binding properties contribute to their distinct effects on *G. oxydans* growth and whether the two globins have different functional roles. Future studies will determine whether the heme-binding properties of these globins contribute to the previously observed effects of HbO on *G. oxydans* growth and whether HbO and HbN have distinct functional roles.

P-03. Methanotroph as a Platform: Methane to Functional Exopolysaccharides for Nanogel Synthesis

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Abstract

Methane (CH_4) is a potent greenhouse gas that can also serve as a low-cost carbon feedstock for microbial production of value-added biomaterials. Methanotrophic bacteria oxidize methane through methane monooxygenases, including the copper-regulated particulate methane monooxygenase (pMMO). This study investigated copper-dependent regulation of methane oxidation in a Methylocystis sp. isolate and evaluated methane-derived exopolysaccharides (EPS) as precursors for nanomaterial development. The isolate was cultivated in nitrate mineral salts medium with methane as the primary carbon source under different copper concentrations. Methane consumption, oxygen and nitrogen profiles were monitored, while expression of multiple pmoA, pmoB, and pmoC gene copies was evaluated by RT-PCR. EPS produced under copper-free conditions was recovered by ethanol precipitation and characterized using FTIR, nanoIR3/AFM, and scanning electron microscopy. Methane oxidation persisted across copper conditions, while individual pmo gene copies showed differential expression, indicating copy-specific regulation of pMMO. The isolate also produced measurable EPS from methane-derived carbon. FTIR identified hydroxyl, carbonyl, and glycosidic/ether-associated functional groups, while nanoscale characterization revealed a heterogeneous, rough polymeric morphology. These findings demonstrate methane conversion into functional EPS and establish a proof-of-concept platform for producing renewable nanogel precursors from methane, with potential applications in drug delivery and sustainable biomaterials.

P-04. From Genomes to Biopolymers: Low-Cost Recovery and Scalable Production of Extremophile Exopolysaccharides

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Abstract

Exopolysaccharides (EPS) produced by thermophilic bacteria possess high thermal stability and unique functional properties, but traditional extraction methods relying on organic solvents like ethanol remain expensive and challenging to scale. This study presents a cost-effective, alcohol-free recovery strategy using high salt concentrations for EPS precipitation. Experimental optimization showed that increasing salt concentration significantly improved EPS yield compared to standard ethanol precipitation methods. Scanning Electron Microscopy (SEM) and nanoIR3-AFM analyses confirmed dense, aggregated biopolymer morphologies. Machine learning predictive modeling was implemented to analyze the interactive effects of culture parameters—such as temperature, pH, duration, and potassium nitrate concentration—on biopolymer production. To transition from lab-scale extraction to scalable production, a theoretical jacketed reactor design was developed, integrating methane and

oxygen nanobubble technology via a centrifugal pump to enhance gas-liquid mass transfer. Combined with automated controls for pH, temperature, and working volume during semi continuous operation, this integrated approach provides a sustainable and scalable framework for high-yield extremophile EPS production and recovery.

P-05. Microbial Exopolysaccharides as Sustainable and Selective Biosorbents for Lithium Recovery

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Abstract

The increasing demand for lithium has highlighted the need for extraction technologies that are more sustainable, environmentally friendly, and selective than conventional chemical processes, which often require harsh reagents and exhibit poor discrimination against competing ions. This research investigated biological approaches to lithium extraction using exopolysaccharides (EPS) produced by microorganisms as naturally derived adsorbent materials. EPS from extremophilic thermophiles and halophiles were evaluated for their lithium adsorption potential, while EPS produced by *Bacillus licheniformis*, a widespread mesophilic bacterium, served as a comparative model due to its robust EPS production and ease of cultivation. Adsorption studies examined the effects of lithium concentration, contact time, pH, and competing cations; such as sodium, potassium, magnesium, and calcium; on lithium uptake. In parallel, this work explored chemical functionalization strategies to enhance EPS from *Geobacillus* sp. performance by investigating phosphorylation, oxidation, and nanogel synthesis using model polysaccharides as cost-effective and versatile analogs for future EPS modification. These studies establish a framework for developing biologically derived lithium adsorbents and identify promising directions for future research, including evaluating a broader range of competing ion concentrations, investigating stacked adsorption conditions that combine multiple environmental variables, and expanding a library of EPS from diverse microorganisms for comparative performance analyses.

P-06. Antagonism: Understanding the Antagonistic Relationship Between *Bacillus pseudomycoides* and *Burkholderia ambifaria*

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Abstract

Bacillus found in soil are not large contributors to the overall microbiome; however, when isolated from the soil, these bacteria tend to form large, fast-growing colonies that make them appear to be more abundant than they really are. This observation led us to the hypothesis: *Bacillus*, specifically *B. pseudomycoides*, is suppressed by antagonists in soil preventing their rapid growth. We identified potential antagonists in soil by streaking them against *B. pseudomycoides*. Focusing on the gram-negative bacteria, we identified four promising isolates, all being *Burkholderia* species. After developing a method to separate the antagonists from the *Bacillus*, our current research seeks to explain, “What is the growth-inhibiting effect of *Burkholderia ambifaria* on *B. pseudomycoides*?” To examine the antagonistic effects of *Burkholderia ambifaria*, we cultured *B. pseudomycoides* and antagonist samples together on R2A agar which indicated through a clear zone that *B. ambifaria* suppressed the growth of *B.*

pseudomycoides. Blue fluorescent protein (BFP) encoded *Burkholderia* antagonists, a live-dead stain, and confocal microscopy were then used to assess cell viability. The results indicated that the *Burkholderia* swam through the solid agar to reach *B. pseudomycoides* suggesting that there must be cell contact for suppression to occur. This experiment also suggested that due to the lack of dead *B. pseudomycoides* cells surrounding the *Burkholderia* under confocal microscopy, the mechanism of antagonism is one of inhibiting growth versus killing cells. Future experiments will be conducted to further understand the translocation of *Burkholderia* across agar and the specific mechanisms in which *B. ambifaria* suppress *Bacillus*.

P-07. Developing an RB-TnSeq Approach to Study *Methylobacterium extorquens* Plant Colonization under Salt Stress

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Abstract

Soil salinization is an increasing threat to agricultural productivity and food security worldwide. Plant growth-promoting bacteria can improve plant performance under salt stress, but these benefits depend on successful colonization of stressed plants.
Identifying bacterial genes involved in plant colonization under stress could support the development of microbial strategies for improved colonization and stress mitigation, aiding sustainable agriculture. Random Barcode Transposon Sequencing (RB-TnSeq) is a powerful tool for genome-wide fitness analysis, yet existing workflows are largely optimized for culture-based experiments rather than plant-associated environments.
We are establishing an RB-TnSeq workflow to investigate the genetic mechanisms that enable the plant growth-promoting bacterium *Methylobacterium extorquens* to colonize salt-stressed plants. Using *Medicago* as a model host, seeds served as the inoculation platform. Bacterial attachment to seeds was quantified by CFU enumeration to identify inoculum densities that maintained adequate representation of the RB-TnSeq mutant library. Plant growth conditions, including growth duration, growth systems, and media, were evaluated to support bacterial colonization while enabling recovery of sufficient bacterial populations from roots and leaves. Conditions were selected to preserve mutant library representation across multiple bacterial generations and generate adequate genomic DNA for downstream barcode sequencing. In parallel, NaCl concentrations were evaluated to establish salt-stress conditions that challenged both plant growth and bacterial fitness while maintaining sufficient bacterial recovery and plant viability.
This workflow provides a foundation for future RB-TnSeq studies of *M. extorquens* fitness during plant colonization under salt stress and may be adapted to investigate microbial responses to other environmental stresses *in planta*.

P-08. Cultivating Targeted Bacteria from Wastewater Treatment Plants for Antibiotic Discovery

Maria Fernanda Galo Garcia

Abstract

Antibiotic resistance is a growing global health problem that has increased the need to discover new antimicrobial compounds. Historically, antibiotics have been discovered from microorganisms found in natural environments; however, many microbial communities remain unexplored. Wastewater treatment plants contain diverse bacterial communities with potentially valuable biosynthetic capabilities. This project investigated the cultivation of microorganisms found in wastewater, which had been previously identified to be linked to biosynthetic gene clusters (BGCs) through genomic analysis. Bacterial groups associated with

selected BGCs were targeted for laboratory cultivation using different growth conditions and low-nutrient media, including R2A, 10% TSA, and PYT80. Recovered isolates were screened for antimicrobial activity, and isolates of interest were further characterized using 16S rRNA gene PCR and sequencing.
After culturing and identifying bacterial isolates, the organisms recovered from the cultured samples did not overlap with the targeted organisms in the provided genomic dataset. This suggests that the growth conditions tested did not recover the specific organisms identified through genomic analysis. Also, antimicrobial activity was not detected during the initial screening after approximately one week of incubation, but several isolates demonstrated zones of inhibition after one month. The extended incubation period may have allowed slow-growing bacteria to reach sufficient growth or metabolic activity for antimicrobial activity to become detectable.
Although the antimicrobial activity observed was not from the targeted organisms, still, it provides opportunities for untargeted bioprospecting. Future work will focus on optimizing growth conditions, completing 16S sequencing, further characterizing active isolates, and exploring alternative cultivation approaches for difficult-to-grow bacteria.

P-09. Host-associated strain variation in nitrogen metabolism in *Limosilactobacillus reuteri*

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Abstract

The common vertebrate gastrointestinal bacteria *Limosilactobacillus reuteri* is a commensal microbe with important roles in host health and fermentation processes, making it of interest as a probiotic. Notably, subgroups within the *L. reuteri* species are strongly associated with specific host species, including rodents and poultry. We hypothesize that strains of *L. reuteri* from different hosts differ in their use of nitrogen sources, an essential element. We first explored this hypothesis using comparative genome analysis using Roary, with functional annotation through comparison with protein family databases (*n* = 344 public high-quality genomes). These analyses were used to characterize core and accessory gene content and investigate differences in gene content among strains from different host species. We found strong clustering by host in terms of both phylogenetic signal and gene presence/absence across genomes. Variation in genes involved in amino acid biosynthesis was particularly prominent, with these genes occurring more frequently in strains isolated from cattle and less frequently in strains from swine and non-human primates. Secondly, we are optimizing chemically defined media to accurately assess the metabolic pathways of strains of *L. reuteri* from different hosts. We performed literature analysis and biosynthetic pathway analysis to develop a chemically defined media that supports growth of *L. reuteri* strains isolated from human, rodent, poultry, and ruminant hosts. Establishing a working chemically defined medium, combined with genomic analysis, will provide a foundation for studying the response to and utilization of different nitrogen sources by *L. reuteri* under nitrogen-limited culture conditions. Understanding how *L. reuteri* establishes in hosts can help inform the development of probiotic strains that are better suited for different host environments.

P-10. *vgrG* Expression in *B. cepacia* under Various Environmental Conditions

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Abstract

Burkholderia cepacia is a Gram-negative bacterium encoding multiple Type VI Secretion Systems (T6SS). *B. cepacia* can infect plants and humans, which provide very different

environmental conditions for the bacterium and may impact the expression of the T6SS. VgrG proteins are critical components of the T6SS, but the conditions that control gene expression of these proteins in *B. cepacia* have not yet been elucidated. In this project, we investigated the expression of genes predicted to encode these VgrG proteins. The *B. cepacia* genome has a total of 19 putative VgrG-encoding genes, 14 of which we examined here. Reporter plasmids containing *vgrG* promoters fused to the β-galactosidase gene were generated and inserted into the *B. cepacia* genome. We tested gene activation at different temperatures and using different culture methods with qualitative plating assays and quantitative β-galactosidase assays. Two temperature points were tested to simulate the different environments *B. cepacia* can be found: 30°C and 37°C. Overnight samples were grown in both liquid cultures and on LSLB agar plates at these different temperatures to explore the impact of culture methods. Based on these tests, we determined that seven of the tested genes have measurable levels of expression. These seven genes were expressed in all conditions tested. However, higher levels of expression were found when the cultures were grown on plates and when the cultures were grown at 37°C. In the future, we will continue to explore conditions under which the other promoters may be activated and will characterize the downstream protein production.

P-11. *vgrG* Gene Expression in *Burkholderia cepacia* during Interbacterial Competition

Noah Koerselman, Noah Koerselman, and Mitchell Friesz

Gustavus Adolphus College

Abstract

Burkholderia cepacia is a Gram-negative bacterium found in soil that infects plants and humans with cystic fibrosis. It uses a Type VI Secretion System (T6SS) to interact with neighboring cells, with the VgrG protein forming an important part of the system's spike. *Pseudomonas aeruginosa* and *Escherichia coli* also possess a T6SS, however, *P. aeruginosa* uses the system more effectively in bacterial competition than *E. coli*. In the work described here we examined the expression of 14 of the 19 putative VgrG-encoding genes in the *B. cepacia* genome. We created reporter plasmids that contained VgrG promoters upstream of the β-galactosidase gene and inserted these constructs into B. *cepacia.* We tested gene activation of cultures on their own and during competition with other bacteria using qualitative plating assays and quantitative β-galactosidase assays. *B. cepacia* cultures were grown in competition with *Pseudomonas aeruginosa*, *Escherichia coli,* or wild type *B. cepacia* to determine how gene expression changes when other bacteria with T6SSs are present. Seven of the 14 genes showed measurable baseline activity. Of those seven genes, the levels of activity varied between promoters. Competition showed no change in the presence of WT B. *cepacia*, while increased activity was observed when exposed to both P. *aeruginosa*, and E. *coli*. Although overall activity changed between conditions, the relative activity between promoters remained consistent. In the future, we plan to examine the downstream protein production for each of the seven active genes to gain insight into the functionality of the *B. cepacia* T6SS.

P-12. Bioprospecting from Wastewater Treatment Facilities for Novel Antibiotics

Tavary Un

Abstract

Antibiotic resistance is a growing health concern that occurs when bacteria develop resistance to antibiotics that were once effective in killing them. Therefore, diseases that were once treatable are becoming more lethal. Resistance can be caused by the misuse and overuse of antibiotics. Preventing the spread of resistance and developing new drugs are two ways to address this growing health concern. One source for developing new antibiotics are

environmental bacteria which may naturally produce antimicrobial agents. Researchers have found that wastewater treatment facilities harbor a diverse array of microbial communities. The focus of my research was to use wastewater treatment samples from Minnesota facilities to isolate bacteria and discover novel antibiotics. Bacteria were cultured on Potato Dextrose Agar and screened in a bioactivity assay against 4 safe relatives of ESKAPE pathogens (*B. subtilis, E. coli, E. aerogenes,* and *A. baylyi*) to test for antibiotic activity. Three isolates inhibited growth in all strains except *E. coli*. These 3 isolates were identified using 16S rRNA sequencing and have 99% similarity to *Klebsiella* species. A chemical extraction was done on one of the isolates, and an active chemical compound was found in the aqueous layer using an agar overlay. Future work will include whole genome sequencing and antiSMASH analysis to identify novel biosynthetic gene clusters. This method has the potential to identify interesting novel antibiotics.

P-13. Improving the Survival of Nitrogen-Fixing Bacteria for Biofertilizer Applications

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Abstract

Leguminous plants are vital to agriculture worldwide, requiring a symbiotic relationship with rhizobium bacteria to grow best. These bacteria colonize plant root systems to facilitate nitrogen fixation, with each strain associating with a specific host: *Rhizobium leguminosarum* (peas and lentils), *Mesorhizobium ciceri* (chickpeas), and *Bradyrhizobium japonicum* (soybeans). This project's goal was to investigate how structural stabilizers and nutrient amendments affect rhizobia survival after initial application to peat granules and promote growth 14 days post-inoculation. We hypothesized that structural stabilizers would limit die-off at application and nutrient amendments would support subsequent growth, yielding higher 14-day viable titers for all three strains relative to an untreated control. Growth curves determined the most effective growth medium for the rhizobium strains, which were further characterized through genome sequencing, identifying symbiotic genes and, with the assistance of the Comprehensive Antibiotic Resistance Database (CARD), antibiotic-resistance genes. As predicted by CARD, all three strains were resistant to fosfomycin and vancomycin. Rhizobia survival in peat granules was investigated at two temperatures (23°C and 30°C) with six additive treatments, including PVP with cassava flour, YMB media, AG media, and combinations of glycerol, sodium glutamate, $MgSO_4$, arabinose, and mannitol. Viable qPCR testing assessed bacterial survival on treated granules, while plating of extracted samples revealed that additives also promoted growth of native peat bacteria. The results showed PVP and cassava flour significantly reduced initial die-off for *R. leguminosarum* and *M. ciceri* ($p = 0.001$), while YMB significantly increased granule growth ($p = 0.006$). These findings highlight the potential of targeted additive formulations to enhance rhizobia inoculant viability, informing continued development of peat-based carrier products.

P-14. Examining the Role of Nitrogen Fixation on Biofilm Formation in *Methanococcus maripaludis*

Annika Mattice

Abstract

Methanogens are a group of archaea that are strictly anaerobic, non-pathogenic, and are widespread in oxygen-free environments. Within the group of methanogens is
Methanococcus maripaludis, the model organism used in this study to observe nitrogen fixation and biofilm formation. Nitrogen is essential for all life; however, some organisms don't have access to biologically usable forms of nitrogen like ammonia. Nitrogen fixation is a

process that converts non-usable forms of nitrogen (atmospheric nitrogen, N_2) into usable forms (ammonia) and is done by some organisms such as *M. maripaludis*. The *nif* operon is a cluster of genes controlling nitrogen fixation and is well characterized in both bacteria and archaea. Biofilms are common in many microbes, including methanogens like *M. maripaludis*, and are characterized as surface-attached communities. Biofilms can help support microbial growth and protect cells from environmental stress. The relationship between nitrogen fixation and biofilm formation is unknown in *M. maripaludis.* Wild-type biofilm formation in *M. maripaludis* under N_2-fixing conditions was compared to wild-type biofilms under regular conditions using a crystal violet staining assay and optical density measurement. WT in N_2-fixing conditions grew at a slower rate and formed less biofilm compared to WT in normal conditions. Future directions include transposon insertion into the *nif* region to disrupt nitrogen fixation and creating a Δ*nif* mutant unable to fix N_2, which will allow the role of nitrogen fixation in biofilm formation to be studied further.

P-15. Targeted Isolation of Novel Myxobacteria from Minnesota Wastewater for Antibiotic Discovery

Eric Nowak

Abstract

In recent years, the increasing prevalence of antibiotic resistant pathogens has created a high demand for the discovery of novel antimicrobial compounds. Myxobacteria are gliding Gram-negative predatory bacteria recognized for their production of many structurally diverse secondary metabolites. Yet, Myxobacteria remain severely understudied in environments such as municipal wastewater treatment plants (WWTPs). WWTPs are nutrient-rich environments with intense microbial competition, making them a prime location to mine for novel antimicrobial compounds. Prior metagenomic analysis of a Minnesota WWTP identified *Paraliomyxa*, a genus within the Myxococcota closely related to *Myxococcus*. Metagenomic binning of this lineage revealed a 76 kb hybrid Type I PKS/NRPS biosynthetic gene cluster spanning ten modules, which had no close matches to characterized compounds. These results suggested biosynthetic novelty and provided the motivation to isolate and culture myxobacteria. The present study attempted to isolate and taxonomically verify predatory Myxobacteria from wastewater samples using low-nutrient bacteriolytic media with *Escherichia coli* bait to selectively enrich myxobacterial taxa. Enrichment cultures on Water Agar and VY/2-1/5SWS media produced fruiting-body-like structures and subcultures were verified by 16S rRNA V3-V4 PCR amplification and Sanger sequencing. In opposition to the initial hypothesis, sequencing identified Cyanobacteria rather than the target Myxococcota. This result was likely due to the preferential amplification of the faster-growing contaminant by universal 16S primers. These results show the need for the use of Myxococcales-specific PCR primers and refined selection protocols to isolate slow-growing predatory taxa from WWTPs.

P-16. Potential Epigenetic Adaptation Enabling *Escherichia coli* to Survive Low-Dose Fluoride Exposure Across Generations

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[3]*Deptartment of Chemistry, Biochemistry, and Physics, South Dakota State University, Brookings, SD.*

Abstract

Epigenetic memory allows bacteria to transiently inherit altered physiological states across generations without changes to the DNA sequence. We recently identified epigenetic memory mechanisms that contribute to bacterial survival during low-level antibiotic exposure, raising

the question of whether similar mechanisms promote survival during non-antibiotic stress. We investigated the response of *Escherichia coli* to fluoride, a non-antibiotic stressor with antimicrobial properties and relevance to dental health. Cells were exposed to a range of fluoride concentrations on solid media. At the minimum inhibitory concentration (MIC, 200 mM), no colonies formed. At low concentrations (50-150 mM), a few colonies appeared overnight; additional colonies appeared after 2-3 days and remained viable. At intermediate concentrations (175 mM), most cells were killed, but rare survivors formed colonies initiated, on average, by single cells. At 175 mM, each surviving cell expanded to millions of descendants during continued fluoride exposure, indicating that the fluoride-survival phenotype persisted across many generations. Survivors were propagated without fluoride for several generations and re-exposed to the same fluoride concentrations. These populations exhibited enhanced survival upon re-exposure, yet their MIC values remained unchanged. The inheritance and reversibility of this phenotype are consistent with epigenetic adaptation. Whole genome sequencing is underway to determine whether genetic changes contribute to the phenotype, and survivor cells are being prepared for transcriptomic analyses to identify genes associated with fluoride survival. Comparing these pathways with those identified during low-dose antibiotic exposure will determine whether epigenetic adaptation to antibiotic and non-antibiotic stress uses shared or distinct pathways.

P-17. Potential Epigenetic Adaptation Enabling *Escherichia coli* to Survive Low-Dose Fluoride Exposure Across Generations

Erin Case[1,2], Haley Champeau[1,2], Suchi Parvin Biki[1,2], Nicholas C. Butzin[1,2,3]

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[3]*Deptartment of Chemistry, Biochemistry, and Physics, South Dakota State University, Brookings, SD.*

Abstract

Epigenetic memory allows bacteria to transiently inherit altered physiological states across generations without changes to the DNA sequence. We recently identified epigenetic memory mechanisms that contribute to bacterial survival during low-level antibiotic exposure, raising the question of whether similar mechanisms promote survival during non-antibiotic stress. We investigated the response of *Escherichia coli* to fluoride, a non-antibiotic stressor with antimicrobial properties and relevance to dental health. Cells were exposed to a range of fluoride concentrations on solid media. At the minimum inhibitory concentration (MIC, 200 mM), no colonies formed. At low concentrations (50-150 mM), a few colonies appeared overnight; additional colonies appeared after 2-3 days and remained viable. At intermediate concentrations (175 mM), most cells were killed, but rare survivors formed colonies initiated, on average, by single cells. At 175 mM, each surviving cell expanded to millions of descendants during continued fluoride exposure, indicating that the fluoride-survival phenotype persisted across many generations. Survivors were propagated without fluoride for several generations and re-exposed to the same fluoride concentrations. These populations exhibited enhanced survival upon re-exposure, yet their MIC values remained unchanged. The inheritance and reversibility of this phenotype are consistent with epigenetic adaptation. Whole genome sequencing is underway to determine whether genetic changes contribute to the phenotype, and survivor cells are being prepared for transcriptomic analyses to identify genes associated with fluoride survival. Comparing these pathways with those identified during low-dose antibiotic exposure will determine whether epigenetic adaptation to antibiotic and non-antibiotic stress uses shared or distinct pathways.

P-18. A Screen to Identify Phosphatidylglycerophosphate Phosphatases in *Clostridioides difficile*

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Abstract

Bacterial cell envelopes, and the enzymes required for their synthesis, are key targets for antibiotics. *Clostridioides difficile*'s membrane is largely made up of phosphatidylglycerol and is essential for *C. difficile* survival. The enzymes required for synthesis have largely been identified, except for the phosphatase that is responsible for converting phosphatidylglycerophosphate to phosphatidylglycerol, which until recently, was true of nearly all Gram-positive bacteria. It was recently discovered that PgpP phosphatidylglycerophosphate phosphatase) is essential for phosphatidylglycerol synthesis and survival in *Bacillus subtilis*. Utilizing bioinformatics, we searched the C. difficile genome for this family of phosphatase and identified 19 candidate genes. We used a *B. subtilis* strain in which *pgpP* expression is dependent upon IPTG (in the presence of IPTG this strain grows normally; however, in the absence of IPTG it is not viable). We cloned the candidate *C. difficile* genes under the control of a xylose-induced promoter. Preliminary plating of these 19 constructs showed no restoration in the *pgpP B. subtilis* strain. This suggests either the phosphatases are not expressed or are insufficient for phosphatidylglycerol synthesis. We are constructing a *C. difficile* genomic library where genes will be expressed from a xylose-inducible promoter. We will screen the library for plasmids that restore viability in a *B. subtilis pgpP*-depletion strain. We will sequence complementing constructs to identify putative phosphatase gene(s). Understanding the enzymatic pathway for this essential phospholipid in *C. difficile* could help identify potential antibiotic targets.

P-19. Investigating the immune response of male and female TLR-7 Knockout and Wild-type against inhaled *Aspergillus fumigatus*

Rsan Mustafa

Abstract

A. fumigatus is a common opportunistic airborne fungus that poses significant health risks, especially for those with pre-existing allergic respiratory illnesses and those that are immunocompromised. Toll Like Receptor (TLR)-7 is important for the activation of the innate immune response; however, there is little knowledge against *A. fumigatus* regarding this receptor. Wild-type or TLR-7 deficient male and female mice and were exposed to airborne *A. fumigatus* spores. Bronchoalveolar lavage cells were collected; collagen and mucus were assessed using histological staining. Data was collected from naïve mice and mice at day 1 and day 14 post-exposure. Mucus and collagen levels had increased by day 1 post exposure and did not return to baseline till day 14 post exposure. TLR-7 knockout mice had higher levels of Macrophages, Neutrophils, and Eosinophils. Females had higher level of immune cells and IgE antibody levels overall. The results of this study will inform fungal disease modeling in mice and can help design better therapeutics and diagnostics for future reference.

P-20. Investigating the Role of the Golgi Tethering Complex GARP in Herpes Simplex Virus Type 1 Infection in CAD Cells

Kassandra Alvarez

Abstract

Herpes simplex virus type 1 (HSV-1) is a complex enveloped virus that completes assembly in the cytoplasm after nuclear egress, where capsids acquire tegument proteins and a final envelope containing viral glycoproteins. The membrane source of this envelope remains unresolved, with evidence supporting either the Golgi or endosomal membranes. In neuronal cells, HSV-1 reorganizes these compartments into a cytoplasmic viral assembly compartment (cVAC) that recruits components needed for virion assembly. Within this compartment, HSV-1 induces a stable association between Golgi and endosomal membranes that persists even after nocodazole-mediated microtubule disruption. However, the mechanism by which HSV-1 establishes this stable association remains largely unknown. The Golgi-associated retrograde protein (GARP) complex is a cellular membrane tethering complex that captures endosome-derived vesicles at the trans-Golgi network (TGN), facilitating endosome-to-Golgi transport. We hypothesized that HSV-1 requires the GARP complex to induce stable Golgi–endosome association during infection and to support efficient viral assembly in neuronal cells. To test this, we generated VPS53-knockout (KO) CAD neuronal cells lacking VPS53, a subunit of the GARP complex. We predicted that this association would be reduced in the absence of GARP. Immunofluorescence confocal imaging of nocodazole-treated infected cells was used to visualize Golgi and endosomal membranes. This analysis showed reduced Golgi–endosome association in knockout cells compared with wild-type cells, suggesting that GARP contributes to this microtubule-independent membrane association. Single-step growth assays showed significantly lower viral titers in KO cells than in wild-type cells at 16 hours post-infection. This suggests that GARP promotes efficient production of infectious HSV-1.

P-21. Identification and Characterization of Newly Secreted Effectors in *Orientia tsutsugamushi*

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Department of Microbiology and Immunology

Abstract

Orientia tsutsugamushi (*O.t.*) is a gram-negative, obligate intracellular bacterium and the causative agent of scrub typhus. This is a neglected, emerging disease that has no vaccines and infects about 1 million people annually. *O.t*. likely modulates its host environment using secreted effectors delivered by type 1 secretion systems and type four secretion systems. Predicted effectors in *O.t.* are categorized into two classes that contain common eukaryotic protein interaction motifs: Ankyrin repeat-containing proteins (Anks) and tetratricopeptide repeat containing proteins (TPRs). But identification of secreted effectors during native infection has been hampered by the lack of genetic tools for *O.t.* Anks have previously been shown to be secreted through a T1SS surrogate bacteria in *E. coli* and implicated in effecting host transcription, apoptosis and immune pathways. However, the role of TPRs is largely unexplored. We hypothesize that *O.t*. TRP proteins function as T1SS effectors. Using a *E. coli* hemolysin A secretion system, we demonstrated that the *O.t.* TPRs: KARP_00126, KARP_00341, KARP_00529, KARP_01308 are capable of being secreted. When ectopically expressed in HeLa and EA.hy926 endothelial cells, the secreted TPRs have unique localization patterns. Bioinformatics identified several domains that may bind with components of the anaphase promoting complex (APC/C). Future research will determine if *O.t.* secretes TPRs to manipulate the host cell cycle and why this is beneficial for the bacteria.

P-22. Multifactorial Immune Response Between Male and Female BALB/cJ and C57BL/6J Mice Exposed to *Aspergillus fumigatus*

Jayden Taylor

Abstract

Aspergillus fumigatus is an opportunistic airborne fungus that poses significant health risks, particularly to immunocompromised individuals and patients with preexisting conditions such as asthma. Despite its clinical relevance, the interplay between host sex, genetic background, and immune response to *A. fumigatus* exposure remains underexplored. This study investigated how immune cell counts, mucus production, collagen, and antibody production vary with sex and strain in BALB/cJ and C57BL/6J mice exposed to *A. fumigatus*.
Mice were challenged with airborne *A. fumigatus* spores weekly for three consecutive weeks. Bronchoalveolar lavage (BAL) was performed to collect immune cells, and histological analysis of lung tissue was conducted to assess mucus and collagen production, and an ELISA was conducted to analyze IgE antibody levels. Immune cells were identified using morphometric differences; mucus and collagen production were scored using Periodic Acid-Schiff (PAS) staining and Series Pico-Sirius Red (PSR) with Fast Green (FCF) counterstaining respectively.
Results showed that granulocyte counts peaked at day 3 post-challenge and declined by day 28, with male C57BL/6J mice exhibiting significantly higher granulocyte numbers than male BALB/cJ mice. While immune cell counts were not significantly different between sexes, mucus production varied. Male C57BL/6J mice had higher mucus production on day 3 post-challenge, whereas female BALB/cJ mice showed elevated mucus levels at day 28. Across all time-points females had significantly higher IgE production compared to their male counterpart This study highlights that both genetic background (strain) and sex influence immune responses, mucus, collagen, and antibody production dynamics in *A. fumigatus* infections. These findings have implications for improving fungal disease models and developing sex- and population-specific therapeutic strategies.

P-23. Clostridioides difficile: Searching for New Antibacterial Solutions to an Old Foe

Kristen Maquindang[1,2], Michelle Steyn[1,2], and Nicholas C. Butzin[1,2,3]

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Abstract

Clostridioides difficile (*C. diff*) is a major cause of antibiotic-associated gastrointestinal disease and healthcare-associated infections. Antibiotic exposure disrupts normal gut microbiota, allowing *C. diff* to proliferate and cause disease ranging from diarrhea and colitis to life-threatening toxic megacolon. Disease is mediated primarily by bacterial toxins that damage intestinal epithelial cells and disrupt the intestinal barrier. New therapies are needed because *C. diff* is resistant to multiple antibiotic classes, while current treatments are also limited by high recurrence rates (20-25%) after an initial episode and further disruption of the commensal microbiota that provides colonization resistance. An ideal therapy would be active against *C. diff* while sparing commensal anaerobes. We recently identified 9 novel small molecules with antibacterial activity against clinically relevant bacteria, including ESKAPE pathogens. We continue to identify additional candidates through computational and experimental screening. Here, we aim to determine whether these compounds, particularly those active against Gram-positive bacteria, are also active against *C. diff*, a Gram-positive bacterium, under anaerobic conditions and to establish reproducible conditions for evaluating their antibacterial activity. Because *C. diff* is an obligate anaerobe, susceptibility testing requires oxygen-free cultivation.

We are optimizing growth, inoculum preparation, and antimicrobial susceptibility assays using vancomycin as a reference antibiotic. Initial experiments use solid-media minimum inhibitory concentration (MIC) assays to establish baseline responses before testing compounds. These conditions will then be adapted for higher-throughput screening.

P-24. Strain variation in responses to reactive nitrogen species responses in the common gut microbe *Eggerthella lenta*

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Abstract

Microbes in the human gut are exposed to a wide variety of stressors, including free radical compounds that can cause DNA damage and disrupt respiration. *Eggerthella lenta* is a common gut microbe found in 90% of North American adults. It has a variable pangenome and can play a role in autoimmune and inflammatory disease. A specific lineage of *E. lenta*, known as Group B, is more abundant in individuals with active gut inflammation. In the gut, these bacteria may encounter host-produced antimicrobial compounds as well as antibiotics. The objective of this study is to evaluate the stress response of *E. lenta* to compounds that generate reactive nitrogen species and how these differ between strains. Multiple *E. lenta* strains were exposed to increasing concentrations of metronidazole, an antibiotic that generates reactive nitrogen species, and the host-produced antimicrobial nitric oxide (NO) under anaerobic conditions. Across tested strains (n = 17), Group B *E. lenta* strains were significantly more resistant to NO compared to other strains ($p = 0.015$), based on the median maximum absorbance relative to the control at the highest concentration (128μM). Similarly, Group B strains were also less inhibited by metronidazole at a concentration of 0.5 μg/mL ($p = 0.024$). Two *E. lenta* strains displayed clear transcriptional responses to NO and metronidazole using RNA sequencing. We are also investigating how these responses may be influenced by the presence of reducing agents used to maintain an anaerobic environment. Understanding *E. lenta* responses to antimicrobial stressors may help clarify how gut bacteria survive host immune defenses.

P-25. LOX-1-Dependent Regulation of Dectin-1 may Result in Differential Susceptibility to Fungal Pneumonia.

Madison R. Kafka, Madeline M. Chorazy, Natalia Estrada-Hernandez, Devin Gatica, Fernando Moreira-Santana, Lee J. Quinton, Filiz T. Korkmaz

Abstract

Rationale: Pneumonia is the leading cause of infection-related mortality worldwide. Alveolar macrophages (AMs) are the first line of defense against lung pathogens. LOX-1 and Dectin-1 are both C-type lectin receptors expressed by AMs. LOX-1 protects against inflammation while Dectin-1 elicits an immune response to fungal pathogens. Previous work showed that inhibition of LOX-1 results in Dectin-1 downregulation. Therefore, the goal of this project is to determine if LOX-1 regulates the response to fungal infection through receptor co-regulation.
Methods: MH-S (AMs) cells were treated with oxLDL and lipopolysaccharide (LPS) for 24h. Expression of LOX-1 and Dectin-1 were assessed by flow cytometry. MH-S and bone marrow derived macrophages (BMDMs) were stimulated with heat-killed *Candida albicans* for 24h to assess receptor and cytokine expression by RT-qPCR.
Results: Treatment of MH-S cells with oxLDL and LPS led to downregulation of both intra- and extracellular LOX-1 and Dectin-1. IL-6 induction was measured in MH-S cells post-LPS, while HKCA led to an increase in IL-1 expression and a small increase in IL-6. However, LOX-1 is significantly induced in both AMs and BMDMs following exposure to HKCA, with no

change in Dectin-1 expression, indicating that LOX-1 induction may need to occur prior to Dectin-1 activation.
Conclusion: While LOX-1 and Dectin-1 have well-established roles during lung infection, our data suggest that LOX-1 and Dectin-1 are co-regulated. Future work will determine the specific pathways that lead to LOX-1-dependent regulation of Dectin-1 in macrophages and subsequent response to fungi.

P-26. Investigating *Neisseria gonorrhoeae* amino acid utilization in the context of L-lactate dependent growth

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Abstract

Bacterial pathogenicity relies on metabolism of environmental resources, which in *Neisseria gonorrhoeae* (Gc), includes a high concentration of lactate, glucose, and amino acids found in the genital tract. Previous studies have found that lactate increases Gc growth in the presence of glucose and supports oxidative stress resistance. However, Gc grows slower on glucose than on lactate when it is the sole carbon source, and lactate causes Gc cell death under acidic conditions. Previous studies have found that arginine prevents lactate-mediated killing. We investigated the effects of the amino acids proline and arginine on these effects. When Gc was cultured with L-lactate and proline, we found that growth was rescued from the low levels typical of growth on L-lactate. Arginine did not produce this effect, as growth remained low. This suggests that proline and arginineinduced resistance against the effects of lactate stem from two separate mechanisms. Additionally, lactate has been identified as a signaling molecule for Gc that induces Gc microcolony disruption, which allows it to colonize new areas. We are using time-lapse microscopy to investigate if lactate induced microcolony dispersal is prevented by amino acids, and thus if lactate-induced microcolony dispersal is related to lactate's metabolic effects. Determining Gc's use of amino acids during lactate metabolism and signaling reveals how the detrimental effects of lactate are resolved to promote Gc pathogenicity and survival in the genital tract.

P-27. Immunosurveillance of *Chrysemys picta bellii* Populations in Northeast South Dakota

Diego Nava Remigio

Abstract

Chrysemys picta bellii (Gray, 1830) is a widely distributed and well-characterized turtle species across North America, yet populations inhabiting the Prairie Pothole Region remain poorly studied. Because this glaciated wetland ecosystem is highly sensitive to environmental change, establishing immunological baselines for resident turtle populations is essential for long-term monitoring of population health. This study characterized bactericidal activity of plasma, a proxy for innate immunocompetence, in a Prairie Pothole population of *C. p. bellii* and evaluated its relationship with body condition and sampling season.
Turtles (n = 37) were captured during May and June from Clear Lake, Marshall County, South Dakota. Standard morphometric measurements were recorded, and plasma was collected via venipuncture. Bacterial killing assays were performed using *Escherichia coli* (ATCC 8739), diluted to an average working concentration of 100 CFU/mL. Plasma was incubated with the bacterial suspension for 21 minutes at 28°C, then plated to quantify growth relative to pathogen-only controls. Percent inhibition was calculated per subject and analyzed against sex, size, and Body Condition Index (BCI).
While sex as a biological variable did not significantly impact immunocompetency, we observed significant differences in bacterial inhibition based on BCI and between sampling

periods. These preliminary results provide one of the first immunological baselines for *C. p. bellii* in the Prairie Pothole Region and highlight seasonality as a key consideration for future reptilian immune studies.

P-28. Generating a fluorescent reporter plasmid for evaluating gene expression in *Neisseria gonorrohoeae*

Emily Fox, Aimee Potter
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Abstract

Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection (STI) gonorrhea. Neutrophils are a key component of the innate immune response against *N. gonorrhoeae* (Gc). Gc can survive in neutrophil-rich environments, in part by consuming lactate released by neutrophils as a source of nutrients. Gc consumes lactate using lactate permease and metabolizes it using a set of four different lactate dehydrogenases. The goal of this study is to create a reporter system to study lactate permease and lactate dehydrogenase promoter activity in Gc during phagocytosis by neutrophils. To do this, we are investigating the induction of green fluorescent protein (GFP) in Gc from plasmids constructed in *Escherichia coli* by collaborators in Dr. Joanna Hick's lab at the University of Waikato (New Zealand).
As proof of concept for this system, we exposed a Gc strain constitutively expressing GFP from a plasmid (pGreen) to neutrophils. We were able to observe expression of GFP from the pGreen strain intracellular bacteria. From this, we conclude that a reporter system using GFP will likely be successful.

P-29. Characterization of an MHV68-DR Virus

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Abstract

All herpesviruses express their genes in an ordered cascade during replication. Sequential expression of immediate-early, early, and late viral genes orchestrates the transition from nuclear entry to progeny production. To facilitate our studies with gammaherpesviruses, we are characterizing a murine gammaherpes MHV68 dual reporter virus (MHV68-DR) containing both an early gene reporter (CMVp:mIFP) and late gene reporter (ORF52p:GFP). To characterize this virus, we first analyzed reporter expression using flow cytometry over the course of infection. As anticipated, the mIFP and GFP reporters displayed a kinetic profile following the course of MHV68 infection, with the mIFP early reporter on by 4 hours post infection, and the GFP late reporter on by 8 hours post infection. UV-inactivation of MHV68 virions blocked expression of both reporters. Next, we applied an inhibitor of the viral DNA polymerase, phosphonoacetic acid (PAA), to test the response of the late gene GFP reporter. We confirmed that the GFP signal was diminished in PAA-treated cells compared to untreated, as expected. However, we also observed consequences on the magnitude of early gene expression with PAA treatment. In the future, optimization of the PAA treatment, or application of other inhibitors such as cidofivir, is required. Altogether, our results suggest that MHV68-DR is a useful tool to follow replication kinetics of a model gammaherpesvirus in cell culture.

P-30. From Isolation to Annotation: Characterizing Eight Novel Bacteriophages

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Abstract

Bacteriophages are abundant, with scientists estimating that there are 10^{31} on our planet. Yet only a relatively small number of these have been purified and genetically characterized. Phages can be used to successfully treat infections that are antibiotic resistant and annotated genomes help scientists identify phages that are most likely to be effective against specific clinical isolates. Genome annotation also contributes to a better understanding of phage diversity and evolution. Here we report the isolation and purification of eight novel bacteriophages. Dodo, Tiggy93, WinnieLou, Orpington, and SafetyGuy were isolated on *Microbacterium foliorum* NRRL B-24224 . EmGer13, Nish23, and AmandaPanda were isolated on *Mycobacterium smegmatis* mc²155. Based on genetic similarities, Tiggy93, WinnieLou, and SafetyGuy were all assigned to cluster EF. Orpington was assigned to subcluster EA2, Dodo to subcluster GD1, EmGer13 to subcluster B1, and Nish23 and AmandaPanda to cluster H, subclusters 1 and 2, respectively. We have completed the annotation of Dodo (350 protein-coding genes and 38-tRNA genes), EmGer13 (101 protein-coding genes), Tiggy93 (83 protein-coding genes), WinnieLou (82 protein-coding genes), SafetyGuy (83 protein-coding genes), and Orpington (62 protein-coding genes). We are working on annotating the genomes of Nish23 and AmandaPanda. Together, these newly isolated and annotated phages expand our understanding of phage diversity and genomic evolution while providing valuable resources for identifying phages with potential applications in the treatment of antibiotic-resistant bacterial infections.

P-31. The Writing on the Phage: Discovering Cytotoxic and Defense Genes in Mycobacteriophage Papyrus

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Abstract

Papyrus is a bacteriophage that was isolated on the host, *Mycobacterium smegmatis* mc^2 155 from soil collected in Durban, South Africa. It is classified as a cluster R phage based on genome similarity. Phages in cluster R are believed to be lytic, and we are working to confirm this by attempting to raise Papyrus lysogens. Most of our work has been focused on performing a systematic genetic screen to uncover functions of Papyrus phage gene products for which the function is unknown. Toward this goal, we are cloning each of the 100 genes in Papyrus' genome into the pExTra plasmid. The phage genes are cloned under the control of the inducible promoter pTet, allowing us to overexpress the phage genes when we add the inducer anhydrotetracycline (aTc). After electroporating the pExTra-phage gene plasmids into *M. smegmatis*, we assay each phage gene product individually for two phenotypes: cytotoxicity, the ability to interfere with host cell growth, and defense, the ability to protect the host cell from infection by other phages. We are successfully applying this strategy to phages Island3 (cluster I1), Sbash (cluster I2), and EagleEye (cluster A16), all of which are temperate phages, as well. Screens for cytotoxicity and defense are nearly complete for Island3 and Sbash.

P-32. Sustainable EPS Production from Corn Stover Using Thermophilic Bacterium Integrating Lignocellulose Bioconversion with Applications

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Abstract

The sustainable production of microbial exopolysaccharides (EPS) from lignocellulosic biomass represents a promising strategy for biomedical applications however, metabolic bottlenecks frequently constrain industrial scale yields. This study evaluates the thermophilic bacterium for EPS production using untreated corn stover (0.5%) as a renewable substrate. Given the recalcitrant nature of lignocellulosic biomass, a systematic temporal analysis was performed to establish a baseline linking substrate depolymerization with polymer biosynthesis. Supernatant enzyme activities (cellulase, xylanase, and laccase) were monitored daily to assess lignocellulose degradation efficiency, while total sugars, reducing sugars, and protein-based growth kinetics were quantified to evaluate carbon release and redistribution toward EPS synthesis. These metabolic parameters were correlated with daily EPS yields to determine the optimal phase for maximal polymer production. Peak cellulase and xylanase activities coincided with elevated reducing sugar concentrations and enhanced EPS accumulation, indicating efficient redirection of carbon flux toward polysaccharide biosynthesis, whereas laccase activity suggested oxidative modification of lignin that improved substrate accessibility and overall bioconversion efficiency. Structural characterization of the extracted EPS using Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectroscopy (FTIR), and Brunauer Emmett Teller (BET) surface analysis revealed nanoscale polymer architecture, characteristic polysaccharide functional groups, and a porous, high surface-area morphology, respectively. Collectively, these findings establish a mechanistic framework linking lignocellulose depolymerization, metabolic flux dynamics, and EPS biosynthesis in the bacterium supporting the development of scalable waste to biopolymer platforms for applications in biomaterials, controlled drug delivery and sustainable bioprocessing.

P-33. Bioconversion of Condensed Soy Solubles into Short-Chain Fructooligosaccharides Using *Aureobasidium pullulans* for Sustainable Aquaculture Nutrition

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Abstract

Soy bioprocessing generates substantial quantities of low-value carbohydrate-rich waste streams that are typically directed to terrestrial livestock feed or wastewater disposal. A major limitation to higher-value utilization is the presence of raffinose family oligosaccharides (RFOs), antinutritional factors that impair digestion and nutrient absorption in aquaculture species. Following α-galactosidase treatment, condensed soy solubles (CSS) contain approximately 100 mg/mL sucrose, presenting an opportunity for microbial valorization. This study evaluated the use of *Aureobasidium pullulans* to produce fructosyltransferase (FTase), which converts sucrose into glucose and short-chain fructooligosaccharides (scFOS), prebiotic compounds with enhanced bioactivity compared to inulin-derived FOS. Batch and fed-batch fermentations were conducted in 5-L and 150-L bioreactors to assess process scalability. In 5-L batch cultures, maximum scFOS yield and productivity reached 22.1% (dry

matter basis) and 6.5 g/L.h at a cell density of 3.37×10^7 cells/mL. Upon scale-up to 150 L, yield and productivity decreased to 9.59% and 2.2 g/L.h at 1.34×10^9 cells/mL, suggesting that enhanced oxygen transfer favored biomass formation over FTase production. To assess functional efficacy, fermented CSS was incorporated into Nile tilapia (*Oreochromis niloticus*) diets at inclusion levels of 2-10% (0.05-0.26% scFOS). After 12 weeks in a recirculating aquaculture system, 4% inclusion significantly improved feed conversion ratio and specific growth rate. These findings demonstrate that CSS fermentation with *A. pullulans* enables scalable production of value-added scFOS and supports sustainable, circular bioprocessing solutions for aquaculture nutrition.

P-34. Thermophilic Exopolysaccharide Biomanufacturing: Process Development, Scale-Up, and Product Recovery

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Abstract

Microbial exopolysaccharides (EPS) are versatile biopolymers with emerging applications in agriculture, environmental remediation, and sustainable materials. This study develops a scalable thermophilic bioprocess for EPS production by *Parageobacillus thermoglucosidasius*, integrating fermentation optimization, process intensification, downstream recovery, and circular resource utilization. Shake-flask and controlled bioreactor studies evaluated temperature, pH, carbon availability, carbon-to-nitrogen ratio, and feeding strategy across batch, semi-continuous, and fed-batch operations. Through process optimization and intermittent nutrient feeding, EPS concentration increased from 4.2 to 18.68 g L^{-1}, representing an approximately 4.4-fold improvement in production. Downstream process development focuses on efficient polymer recovery and purification, including ethanol recovery by distillation and reuse during EPS precipitation, reducing solvent consumption and improving process circularity. FTIR and ^{1}H NMR analyses confirmed hydroxyl, carboxyl, and glycosidic functionalities associated with the recovered polymer, while genome annotation indicated a potential GDP-mannose-dependent biosynthetic pathway. Current work is extending the process toward pilot-scale production, alternative agro-industrial feedstocks, and application-driven EPS formulations, particularly for soil conditioning and environmental applications. In parallel, techno-economic analysis (TEA) and life-cycle assessment (LCA) are underway to identify major cost, energy, and material-use drivers and quantify the environmental benefits and tradeoffs associated with scale-up. Together, this integrated approach connects thermophilic fermentation, downstream solvent recovery, polymer characterization, process economics, and environmental performance to establish a pathway toward commercially relevant and circular production of functional microbial biopolymers.

P-35. FlavoNex: Soybean-Derived Biostimulant for Sustainable Agriculture

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Abstract

Nitrogen (N) fertilizer is essential for modern crop production but represents a major and increasingly volatile production cost, while inefficient N utilization contributes to nitrate leaching and other environmental losses. Developing biologically based strategies that improve nutrient acquisition while reducing reliance on synthetic fertilizers is therefore

important for both agricultural productivity and sustainability. Plant-derived signaling molecules can influence interactions between plant roots and beneficial soil microorganisms, providing an opportunity to develop microbiome-directed agricultural inputs. Here, we investigated valorizing soybean processing residues to develop FlavoNex, a sprayable biostimulant designed to enhance beneficial plant-microbe interactions and crop performance. We evaluated FlavoNex bioactivity through a multi-tiered experimental framework integrating seed germination, microbial biofilm formation, soil microcosms, and microbial community analysis. Seed germination assays demonstrated a concentration-dependent response, with 250 μM FlavoNex producing approximately a two-fold increase in germination relative to the untreated control, whereas concentrations above 500 μM inhibited germination, indicating a distinct biological response threshold. UV-Vis-based biofilm assays further showed increased surface-associated microbial biomass following FlavoNex treatment, suggesting enhanced microbial attachment and colonization potential.

Importantly, soil microcosm studies demonstrated that FlavoNex shifted microbial community composition and increased the relative abundance of bacterial taxa associated with biological N fixation and plant growth promotion, including *Bradyrhizobium*, *Paenibacillus*, and other beneficial rhizosphere-associated bacteria. These community-level responses support the hypothesis that soybean-derived metabolites can selectively influence agriculturally relevant components of the soil microbiome. We further evaluated soil-applied FlavoNex for its effects on rhizosphere colonization, plant biomass, and plant N status, linking microbial responses with crop-level outcomes.

Collectively, these findings establish a pathway to convert soybean processing residues into value-added agricultural inputs while showing that naturally derived signaling metabolites can modulate plant-microbe interactions and rhizosphere microbial ecology. FlavoNex provides a foundation for developing next-generation, microbiome-directed biostimulants to improve nutrient acquisition, support more efficient N management, and advance circular bioeconomy approaches for sustainable agriculture.

P-36. Oil Type, Dose, and Tween 80 Synergies for β-Carotene Production in *Rhodotorula mucilaginosa*

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Abstract

Red yeasts such as *Rhodotorula mucilaginosa* offer a sustainable route to natural β-carotene, but yields on low-cost substrates like sugar beet molasses remain low. Oil supplementation may enhance carotenogenesis by supplying fatty acids whose β-oxidation increases acetyl-CoA flux into the mevalonate pathway. However, the individual and combined effects of oil type, supplementation rate, and emulsifier are understudied. We screened eight food-grade oils (canola, crude corn, grapeseed, olive, peanut, refined corn, sunflower, and vegetable) at 0.25, 0.5, and 1% (v/v), with and without 1% (v/v) Tween 80, alongside no-oil controls, in a defined sugar beet molasses medium. β-carotene production in 162 shake-flask cultures (54 treatments × triplicate) was evaluated by a three-way ANOVA with Benjamini–Hochberg-adjusted pairwise comparisons. Oil type had a strong main effect ($P = 2.4 \times 10^{-4}$), whereas supplementation rate ($P = 0.063$) and Tween 80 ($P = 0.58$) were not significant. However, the oil × rate × Tween 80 interaction was highly significant ($P = 1.3 \times 10^{-5}$), indicating that each factor's influence depended on the levels of the others. Vegetable, crude corn, and olive oils produced the highest β-carotene concentrations, particularly at 1% (v/v) with Tween 80, whereas sunflower oil and the no-oil controls yielded the lowest. These results show that β-carotene yield is governed by specific combinations of oil, dose, and emulsifier rather than by any single factor, which underscores the need for factorial optimization of lipid

supplementation. The top-performing treatments are currently being scaled up in 7 L bioreactors.

P-37. Engineering a Cell-Free Biocatalytic Platform for Sustainable Synthesis of Polyhydroxyalkanoate Precursors from Renewable Feedstocks

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Abstract

Conventional microbial production of polyhydroxyalkanoates (PHAs) is constrained by competition between product synthesis and cellular growth, variable metabolism, and complex downstream processing. Reconstructing biosynthetic pathways outside living cells offers greater control over enzyme composition and reaction conditions. This study presents a modular cell-free biocatalytic platform for controlled synthesis of PHA precursors from acetate, enabling direct evaluation of individual enzymatic steps and integrated pathway behavior. Enzymatic modules were heterologously expressed in *Escherichia coli* BL21 using IPTG inducible systems. SDS PAGE confirmed protein expression, with bands spanning approximately 35-80 kDa. Enzyme functionality was evaluated using spectrophotometric assays and high performance liquid chromatography (HPLC). Calibrated standards monitored at 190 and 254 nm established characteristic retention times and peak profiles for quantitative monitoring of reaction progress.

Following validation of individual enzymatic steps, multiple enzymes were combined to assess pathway performance. Early integrated reactions demonstrated measurable activity and detectable downstream intermediate formation. Time course analyses showed relatively high initial reaction rates, followed by upstream intermediate accumulation during prolonged operation, suggesting imbalances in enzyme activity or stability. These observations illustrate the platform's utility for resolving catalytic bottlenecks and kinetic limitations without interference from cellular regulation. Ongoing studies optimize enzyme ratios, reaction conditions, cofactor management, and stability through buffer composition, salts, and stabilizing additives. BioLector XT supports parallel screening and time-resolved monitoring to accelerate optimization. Together, these efforts establish an analytical and experimental framework for controlled PHA precursor synthesis from acetate and provide a foundation for further development of flexible, sustainable biomanufacturing systems. Future work will address sustained performance and scalability.

P-38. Process Optimization and Hybrid Nanoflower Synthesis of 5-Hydroxymethylfurfural Oxidase for efficient oxidation of HMF and its derivatives to 2,5-furandicarboxylic acid

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Abstract

2,5-furandicarboxylic acid (FDCA) is a precursor of the biopolymer Polyethylene furandicarboxylate (PEF). 5-hydroxymethylfurfural oxidase (5-HMFO) oxidises 5-hydroxymethylfurfural (HMF) to FDCA. One such promising biocatalyst is 5-HMFO from Pseudomonas poae (PSPO). The major limitations of free enzymes include high cost, low thermal stability, limited storage stability and lack of reusability. To mitigate these limitations, the protein-inorganic hybrid nanoflowers of PSPO and Mn^{2+} (PSPO@MnNF) was developed.

PSPO@MnNF exhibited a flower-like morphology, as observed under scanning electron microscopy. Molecular dynamics simulations were conducted to elucidate the coordination patterns and binding interactions between PSPO and the Mn^{2+} and Co^{2+} ions. PSPO was upscaled in a 5 L fermenter yielding 1.004 ± 0.2 g/L of recombinant protein. Analytical and biochemical characterization of hybrid nanoflowers showed enhanced storage stability, improved thermostability, higher catalytic efficiency, retaining more than 95% activity after the fifth reaction cycles for the substrate vanillyl alcohol and more than 80% activity after two cycles for the substrate HMF. At 4 ºC, PSPO@MnNF maintained 95% of its residual activity during 30 days of storage. After immobilization, the kinetic parameters of PSPO@MnNF displayed lower Km for the substrates HMF, FFCA, DFF and vanillyl alcohol (1.620 mM, 2.309 mM, 0.675 mM and 0.792 mM, respectively) compared with free PSPO (2.252 mM, 5.551 mM, 2.503 mM and 1.589 mM, respectively). Hence, the developed PSPO@MnNF could be an efficient biocatalyst for the sustainable oxidation of HMF, FFCA, DFF and vanillyl alcohol.

P-39. Antifungal Activity of *Burkholderia* Against Cheese Spoilage Fungi

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Abstract

Fungal growth on the surface of packaged cheese is a common spoilage problem that can affect product quality, shorten shelf life, and cause economic losses. Methods such as heat treatment, refrigeration, and chemical preservatives are often used to control microbial spoilage, but they can have drawbacks. For example, heat processing may affect the flavor or nutritional quality of cheese, and there is growing interest in finding safer biological alternatives. In this study, we tested whether compounds released by a bacterium could suppress fungi associated with cheese spoilage. A soil isolate identified as *Burkholderia* strain BYP_9 was evaluated for antifungal activity. Fungi were isolated directly from cheese by homogenizing samples in sterile water and plating them on potato dextrose agar. Three dominant isolates were isolated and identified by ITS sequencing as two *Penicillium* species and one *Candida zeylanoides*. Antifungal activity was tested using agar well diffusion assays. When 300 µL of cell-free supernatant from BYP_9 cultures was added to fungal lawns, clear inhibition zones were observed against all three isolates. Live/dead staining also showed reduced fungal cell viability and lower cell biomass after exposure to the supernatant compared to the control, suggesting a suppression effect. Genome analysis of BYP_9 identified nine known biosynthetic gene clusters that may be involved in antimicrobial metabolite production. Overall, these results suggest that extracellular metabolites produced by BYP_9 can suppress cheese spoilage fungi and may have potential as natural biocontrol agents for enhancing shelf-life. This approach could offer a practical alternative to conventional cheese preservation methods.

P-40. Phenolic Compounds from Camelina Meal Exhibit Antiglycation Properties Through Complementary Mechanisms

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Abstract

Chronic diseases such as cardiovascular disease and diabetes are leading causes of death globally. An increasing number of studies have indicated a link between advanced glycation end-products (AGEs) and chronic diseases. While synthetic molecules, such as

aminoguanidine, can inhibit AGE formation, their associated side effects make their use impractical. This situation has therefore prompted increased research interest in identifying natural sources of AGE-inhibiting molecules. In this study, we examined the AGE-inhibitory activities of cold-pressed camelina meal (CPCM) extract. The 80% methanolic extract of CPCM showed notable bioactivity, with total phenol content of 44.61 ± 2.79 mg GAE/g extract. Its antioxidant activities were 304.37 ± 4.01 µmol Trolox equivalents/g extract (ABTS assay), and 368.14 ± 21.59 µmol Trolox equivalents/g extract (DPPH assay). UHPLC-HESI-Orbitrap MS^n profiling identified major phenolics (sinapine, kaempferol 3-*O*-rutinoside, and isorhamnetin 3-*O*-rutinoside) and the glucosinolates (glucoarabin, glucocamelinin, 11-(methylsulfinyl)undecyl-glucosinolate) in the methanolic extract. Pure phenolics exhibited higher antiglycation potencies than AG, with flavonol glycosides displaying ~9-12-fold lower IC_{50} values, whereas glucosinolates were inactive. The mechanistic assays suggested that the extract inhibits AGE formation by partially inhibiting the formation of fructosamine adducts and α-dicarbonyl compounds, and chelating metals (pro-oxidant). These results indicate cold-pressed camelina meal is a valuable source of natural AGE inhibitors. Further isolation and characterization of these high-value bioactive compounds may support its application in nutraceutical formulations and contribute to the value-added utilization of camelina meals.

P-41. Reducing Gas Flux during Ruminal Fermentation with Statin-Enriched Corn Ethanol Co-Products

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Abstract

Enteric gas emissions from ruminant animals represent a considerable waste of feed energy and a possible environmental hazard for animal agriculture. Lovastatin, a secondary metabolite of *Aspergillus terreus*, inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a critical enzyme in archaeal membrane lipid synthesis, and suppresses methanogenic archaea that produce gas in the rumen. The purpose of this study was to assess the possibility of lovastatin-enriched corn ethanol co-products as a sustainable feed addition for reducing ruminal gas flux while preserving overall microbial community stability. To generate lovastatin-enriched biomass, solid-state fermentation of corn wet cake was performed using *Aspergillus terreus*. Fermented products were evaluated utilizing in vitro rumen fermentation systems based on rumen fluid and manure. The treatments included Lovastatin doses ranging from 1 mg to 100 mg and incubation times (24, 48, 72, 96, and 120 hours).16S rRNA sequencing and taxonomic categorization were utilized to investigate the microbial community's reactions. The relative abundance of archaeal populations was compared between treatments and controls. Lovastatin reduced methanogenic archaeal abundance in a dosage and time-dependent manner. Longer incubation times resulted in the steepest decreases, indicating long-term methanogenic population suppression. However, the general bacterial community remained rather steady, indicating a selection influence on the archaeal groups under consideration. The results show that using upcycled corn ethanol co-products is a potential technique for reducing ruminal gas flux. Quantification of gas production dynamics, assessment of fermentation end products, and in vivo efficacy of statin-enriched co-products are ongoing and will be discussed.

P-42. Reconstitution of the Hevea Rubber Elongation Complex in *Pichia pastoris* for Membrane Directed Natural Rubber Biosynthesis

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Abstract

Natural rubber is a high-value biopolymer whose biosynthesis remains difficult to reproduce outside its native plant system because it depends on a membrane-associated, multienzyme complex rather than a single soluble catalyst. Synthetic rubber enables large-scale production but has limitations in mechanical performance and relies on petrochemical feedstocks, raising sustainability and supply concerns. Natural rubber production is also highly dependent on *Hevea brasiliensis*, a geographically restricted crop vulnerable to climate variability, supply-chain disruption, and pathogens such as South American Leaf Blight. These challenges motivate scalable, geographically flexible microbial production.

Rubber biosynthesis in *H. brasiliensis* involves the coordinated HRT1-HRBP-REF-SRPP complex at a specialized lipid interface, where isopentenyl pyrophosphate (IPP) is polymerized into high-molecular weight cis-1,4-polyisoprene. Current heterologous systems have not reliably reproduced this product profile, suggesting that successful reconstruction requires coordinated expression, membrane localization, protein folding, and complex assembly.

This work develops *Pichia pastoris* as a eukaryotic chassis to reconstruct the HRT1-HRBP-REF-SRPP system. Golden Gate modular cloning, codon optimization, promoter selection, and membrane-targeting strategies are used to generate individual and combinatorial constructs. Cloning and transformants are evaluated by molecular analysis, while SDS-PAGE, Western blotting, and confocal fluorescence microscopy assess protein expression and localization. Subsequent studies will investigate protein interactions and complex assembly using FLIM-FRET, co-immunoprecipitation, and crosslinking, alongside membrane characterization through fluorescence. Polymer formation will be evaluated using GC-MS, LC-MS, SEC, NMR, DSC, and TGA to assess composition, molecular weight, and material properties. Overall, this study establishes a microbial membrane-based platform for reconstructing natural rubber biosynthesis and provides a foundation for scalable production of high-quality natural rubber.

P-43. Cohesive fungal monitoring systems demonstrate crop protection insight

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Abstract

The use of reduced tillage and no-till in farming operations is becoming more common in the United States, and South Dakota is no exception. Right now, there is little information on the pathogen pressure present in most areas. To remedy this, the National Predictive Modeling Tool Initiative (NPMTI) has increased efforts to collect data on common wheat pathogens using samples from wind currents and plant debris in wheat fields across South Dakota. With this data, NPMTI plans to determine a baseline pressure for prevalent diseases and create a forecast system for potential outbreaks. The most prevalent foliar wheat fungal diseases in South Dakota include Leaf Rust, Stem Rust, Stripe Rust, Tan Spot, Septoria Nodorum Blotch, and Septoria Leaf Blotch. Samples were collected from spring and winter wheat fields from 2021 to 2025. Of the 2,035 samples collected, over 600 were residues, and 1,300 were from air collections. Septoria Nodorum Blotch and Tan Spot were the most commonly occurring diseases, while Stem Rust and Septoria Leaf Blotch levels were higher in years with more precipitation. The most abundant CQ values were in 2025 compared to any other year, likely due to the higher precipitation level creating more desirable conditions for disease development. The greatest CQ values came from residue samples. Stem Rust, Stripe Rust, Septoria Nodorum Blotch, and Tan Spot showed statistically significant differences in recorded levels between residue and air samples, indicating they may have higher levels or be easier to track with one method compared to the other.

P-44. Management of Internal Rot of Peppers

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Abstract

Internal Rot of Peppers is a poorly understood disease that results in pepper fruit rotting from the inside out during fruit maturation. This disease is caused by several fungi, the most common of which is *Alternaria alternata.* This study seeks to understand the factors that favor disease development, determine varietal susceptibility and develop several management strategies. Disease incidence is higher early in the season and appears linked to humidity. While *Capsicum chinense* varieties are the most susceptible, *C. frutescens* varieties are the most resistant. Additionally, a conventional fungicide program was able to reduce disease rates by more than 50%. These findings will help guide management of internal rot in peppers.

P-45. A Global Microbiome Meta-analysis of Soil Microbial Responses to Plant Invasion

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Abstract

Plant invasion alters soil microbiota and ecosystem processes through soil-mediated feedbacks. However, reports of its influence on soil microbial taxonomic diversity remain inconsistent, and corresponding changes in functional diversity remain poorly understood. We conducted a global microbiome meta-analysis comparing invasive and native plant-associated

bacteria and fungi across 3,621 samples from 35 studies spanning 16 countries, six continents, and four plant functional types to test whether taxonomic changes align with functional reorganization or indicate taxonomic-functional decoupling. We found that invasive plants harbored significantly higher taxonomic diversity than native plants for soil bacteria ($p<0.001$) and fungi ($p<0.001$), with consistent patterns across continents and plant functional types. However, functional diversity exhibited opposite trends with invader-associated communities, being lower than native ($p<0.01$), suggesting a taxonomic-functional decoupling. This decoupling was particularly strong for invasive forbs in Asia ($r =-0.34$) and North America ($r=-0.33$) and for invasive grasses in North America ($r =-0.21$). FAPROTAX profiling revealed that microbiomes of native plants harbored diverse functional repertoires such as photoautotrophy, nitrification, whereas invasive communities exhibited functional specialization, like aromatic hydrocarbon degradation. Fungal trait analysis further showed enrichment of ectomycorrhizal and root-endophytic lifestyles under native plants, while plant-pathogenic lifestyles were enriched under invasive plants. Our ensemble network analysis, utilizing Spearman, MIC, and FlashWeave algorithms, revealed contrasting organizational patterns: bacterial networks associated with invasive plants showed greater mean degree and connectivity, whereas fungal networks showed greater connectivity under native plants. Overall, our findings demonstrate that plant invasion redefines biodiversity-function relationships in soil microbiomes, promoting taxonomic expansion alongside functional specialization globally.

P-46. Empirical Validation of Keystone Taxa in Stressed Synthetic Microbial Communities

Avery Folsom

Abstract

Keystone taxa can have disproportionate effects on microbial community structure and function, yet experimentally validating these roles remains challenging. Network-based approaches can identify candidate keystones, but experimental validation is needed to distinguish true ecological importance from spurious correlations. Here, we combined synthetic microbial communities (SynComs) with multi-omics approaches to validate candidate keystone taxa and investigate traits that may contribute to their ecological roles. We first assembled an 82-member SynCom from bacterial isolates collected from highly saline soils (10 dS/m) at the Langdon Research and Extension Center in North Dakota, USA, and used network analysis across salinity treatments to identify candidate keystones. We then constructed a reduced 20-member SynCom containing three species-level candidates, *Arthrobacter oryzae*, *Rossellomorea marisflavi*, and *Sphingobium xenophagum*, that overlapped between field and SynCom networks. Targeted dropout communities were created by removing keystones individually and in combination. Whole-genome sequencing was performed for all three taxa, along with RNA-Seq of *A. oryzae* across salinity conditions. Removing all three keystones significantly reduced network connectivity, while individual dropouts identified *A. oryzae* as the strongest contributor to community structure. Genomic analyses indicated enrichment of disordered protein regions and regulatory pathways, suggesting metabolic flexibility. Transcriptomic analysis of *A. oryzae* showed salinity-associated metabolic streamlining, including increased expression of membrane proteins, chaperones, ATP synthesis, and translation-related functions. Together, these results provide a framework for species-level validation of keystone taxa and demonstrate their disproportionate influence on SynCom resilience.

P-47. Thermophilic Nitrogen-Fixing Cyanobacteria from Yellowstone National Park as a Platform for Sustainable Biohydrogen Production

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Abstract

Modern agriculture depends on nitrogen to sustain a rapidly growing global population, yet the annual production of over 180 million metric tons of synthetic nitrogen fertilizer is highly energy intensive and contributes substantially to greenhouse gas emissions. Continued reliance on fossil fuel driven nitrogen production intensifies climate change and highlights the urgent need for sustainable alternatives. Biological nitrogen fixation offers such an alternative, operating under ambient conditions while naturally producing hydrogen as a byproduct. However, thermophilic nitrogen fixing systems remain largely unexplored, and thermostable nitrogenases have not been reported in cyanobacteria. This work investigates thermophilic nitrogen fixing cyanobacteria isolated from geothermal hot springs in Yellowstone National Park. Cyanobacteria are uniquely suited for this purpose because they are photosynthetic, using sunlight to power nitrogen fixation, enabling carbon neutral biohydrogen production. Extreme thermal environments (60-75°C) naturally favor microorganisms that remain stable and functional under intense heat, positioning them as promising platforms for industrial biotechnology. We hypothesize that thermophilic cyanobacteria exhibit enhanced nitrogenase stability at elevated temperatures, supporting sustained nitrogen fixation and increased hydrogen evolution. Thermophilic strains were isolated and characterized for growth, nitrogenase activity, and hydrogen production. Nitrogen fixation will be Modern agriculture depends on nitrogen to sustain a growing global population, yet the annual production of synthetic nitrogen fertilizer is highly energy-intensive and contributes substantially to greenhouse gas emissions. Continued reliance on fossil-fuel–driven nitrogen production intensifies climate change, underscoring the need for sustainable alternatives such as biological nitrogen fixation, which operates under ambient conditions and generates hydrogen as a natural byproduct; however, thermophilic systems and thermostable cyanobacterial nitrogenases remain largely unexplored.

This study focuses on isolating thermophilic nitrogen-fixing cyanobacteria from geothermal environments in Yellowstone National Park. We hypothesize that cyanobacteria adapted to elevated temperatures (60–73°C) exhibit enhanced nitrogenase stability and sustained metabolic activity, enabling efficient nitrogen fixation coupled with hydrogen evolution under thermophilic conditions. Enrichment and isolation efforts were conducted at 60°C, 65°C, and 73°C to target organisms adapted to high-temperature environments. Media composition was optimized using geochemical modeling with MINTEQ software to simulate thermal spring chemistry and improve thermophilic growth.

Successful growth was observed at elevated temperatures, and isolates were evaluated for purity using light microscopy. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was performed to analyze trace metal composition relevant to nitrogenase cofactors. Nitrogenase activity will be assessed using the acetylene reduction assay, and ongoing genomic analysis will be performed to identify nif gene clusters associated with nitrogenase systems. Our findings demonstrate successful isolation of thermophilic nitrogen fixing cyanobacteria capable of sustained activity at high temperatures, supporting their potential as a platform for renewable hydrogen generation.

These findings establish foundational progress toward thermophilic biological nitrogen fixation linked to biohydrogen production. Future work will optimize nitrogenase activity, enhance

hydrogen yield, and further characterize thermostable systems. In parallel, we are developing **Cyanovault**, a curated repository to catalog and preserve thermophilic cyanobacterial isolates to support extremophile-based biotechnology research. This work advances extremophile-based strategies for sustainable nitrogen management and clean energy production.

P-48. Genomic and Phenotypic Study of *Bacillus velezensis* Isolates Having Biological Control and Plant Growth-Promoting Activities Benefiting Wheat

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Many *Bacillus amyloliquefaciens* strains are plant growth-promoting rhizobacteria that can also act as biological control agents targeting plant pathogens. We obtained two isolates of this species, 1BA and 1D3, from wheat residue and tested them for antifungal activity against two cereal pathogens, *Fusarium graminearum* and *Pyrenophora tritici-repentis*. Whole-genome sequencing revealed that both isolates we had previously identified as *B. amyloliquefaciens* were currently classified as *Bacillus velezensis*. Comparative genomic analysis targeting genes for surfactin biosynthesis, auxin-related pathways, and volatile organic compounds (VOCs) showed they were present in both isolates. Both had nearly genetically identical (99.99%) genomes of 3.98 Mb and lacked the iturin and fengycin gene clusters. However, both possessed a non-ribosomal lipopeptide biosynthetic gene cluster responsible for plipastatin production, which exhibits antifungal activity. Functional annotation further identified genes encoding xylanase, multiple proteases and lipases, cellulase, alpha-amylase, peroxidases, and bifunctional cytochrome P450, which may collectively contribute to bacterial antifungal activity, plant growth promotion, nutrient cycling, and beneficial plant interactions. Although the two isolates were genetically identical, they exhibited clear phenotypic differences. Isolate 1BA formed raised wrinkled colonies and had faster growth, whereas isolate 1D3 produced colony papillae and had slower growth. Dual culture assays demonstrated that both isolates exhibited more pronounced antifungal activity against *F. graminearum* and *P. tritici-repentis* compared to several other *Bacillus* species. Despite minimal genomic differences, the observed phenotypic variation suggests differential expression or regulation of key traits that may influence plant association, with lipopeptide production and hydrolytic enzyme activity being important for plant association.

P-49. From Seedling to Harvest: Management and Environmental Drivers of Plant-Mycorrhizal Symbiosis

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Abstract

Arbuscular mycorrhizal fungi (AMF) have co-evolved with terrestrial plants, forming symbiotic associations that support plant nutrient acquisition and stress tolerance throughout the development. Although the establishment of AMF symbioses under different nutrient conditions has been extensively studied, how AMF diversity and root colonization change throughout crop development under heterogeneous field conditions remains poorly understood**.** To address this knowledge gap at a regional scale, we investigated AMF communities in spring wheat fields across North Dakota, USA. Root samples were collected across seedling, mature and harvest stages of wheat using 603 root samples from 201 agricultural sites across North Dakota, USA. We integrated AMF profiling with 60,300 microscopic root-intersection observations, soil and weather data, management intensity, host genotype, and the harvest-stage plant ionome comprising macro- and micronutrient composition. Root colonization was quantified by recording intersections containing

arbuscules and hyphae, while AMF community diversity was characterized using 18S amplicon sequencing. Microscopic hyphal colonization was positively associated with sequencing-based Shannon diversity, providing complementary evidence that shifts in root colonization coincided with changes in AMF community structure. Mixed-model analyses further identified growth stage and agricultural management as important components of AMF dynamics. Structural equation modeling revealed direct negative effects of increasing management intensity on both Shannon diversity and hyphal colonization, while soil nutrient stoichiometry and climatic conditions exerted additional direct effects. Together, these findings demonstrate developmental restructuring of AMF communities across the growing season and show that these trajectories are jointly shaped by agricultural management and environmental conditions at a regional scale.

P-50. Bacterial adaptations to lead enrichment in the La Crosse River Marsh

Rachel Zimmerman

Abstract

The La Crosse River Marsh in La Crosse, Wisconsin was a fallout zone for lead shot discharged from four trap shooting stations at the La Crosse Gun Club, resulting in over 31,000 m^3 of sediments exceeding the US Environmental Protection Agency's then-current limit of 400 mg/kg lead. Some climate change scenarios predict worsening drought severe enough to aerially expose these sediments, necessitating remediation to ensure visitor safety. Bacterial bioremediation may be a promising alternative to traditional methods, but it requires characterizing how native bacteria manage lead exposure. Ninety-seven potentially different lead-susceptible and lead-resistant bacteria were isolated from sediments sampled at a depth of 10-20 cm at low- and high-lead marsh sites. X-ray fluorescence quantified lead concentrations of 0.084 and 0.106 mM Pb at the low site, and 20.7, 26.3, and 30.1 mM at the high site. Sanger sequencing of the V3-V4 16S rDNA region established the identity of three susceptible isolates eliciting minimum inhibitory concentrations of 0.15625 (n = 1) and 0.3125 (n = 2) mM $Pb(NO_3)_2$ and two resistant isolates able to grow on plates containing 25 mM $Pb(NO_3)_2$. Scanning electron microscopy with energy-dispersive X-ray spectroscopy was used to detect the presence of lead precipitates. Future work will utilize Inductively Coupled Plasma Atomic Emission Spectroscopy to determine if lead is sequestered within the exopolysaccharide layer and/or internally, and Illumina sequencing will identify resistance genes. Understanding these mechanistic differences will aid in providing the groundwork for establishing effective bioremediation efforts for the marsh.

P-51. Metaomic Analysis and Assessment of Chemolithoautotrophy within Ochraceous Flocs Present in Artesian Well Runoff

Tyler Kaiser

Abstract

Ochraceous flocculent growths consisting of complex macroscopic bacterial blooms encrusted in iron oxides are commonly found where groundwater discharges to the surface. These environments are continuously replenished with dissolved reduced inorganic compounds that are oxidized at the aerobic interface, creating conditions that appear strongly conducive to microbial chemolithoautotrophy. Studying the composition and functional potential of these communities is challenging, however, because of their complexity and the unculturability of many constituent taxa. To address this, we first confirmed the presence of chemolithoautotrophy using a $^{14}CO_3$ uptake assay performed on enrichments supplied with reduced hydrogen, sulfur, or iron. Flocculent material was subsequently flash-frozen and subjected to Illumina sequencing of both the metagenome and metatranscriptome. The $^{14}CO_3$ uptake assays demonstrated that inorganic carbon fixation was dependent upon, and

significantly stimulated by, each of the reduced inorganic electron donors tested. Metagenomic and metatranscriptomic data will further elucidate community composition and the expression of genes linked to their functional traits.

P-52. Effects of MHC Class I Modulation on PAMP-Induced Activation of RAW264.7 Cells

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Abstract

Major histocompatibility complex I (MHC-I), a molecule that sits on all nucleated cells, is a recognized reason for organ and tissue graft rejection. In attempt to understand the possible cell signaling abilities of this molecule, RAW262.7 and THP-I cell lines were stimulated by three different kinds of pattern associated molecular patterns (PAMPS): lipopolysaccharide (LPS), peptidoglycan (PGN), and zymosan (ZYM). Stimulation of our cell lines with different PAMPS is necessary to assess how MHC-I influences different cellular pathways and cytokine production. When macrophages/monocytes are activated, they express more levels of CD80 on the surface. In order to measure this stimulation, we have tagged our cell lines with an anti-CD80 antibody and utilized flow cytometry. Future work will confirm protein expression of a protein downstream of the MAPK pathway, we will stain our cells with an anti-p38 and use western blot analysis. As we are working with a cell line, our model also utilizes MHC-I stimulating antibodies (SF-1, 34-1-2S and IgG control) to mimic stimulation from CD8+ T cells. This is to understand the impact of stimulated MHC-I on cell stimulation. Additionally, we compare MHC-I unstimulated cells to MHC-I stimulated cells and observe activation level differences. A decrease in macrophage/monocyte activation with MHC-I stimulation was observed using LPS and PGN.

P-53. Validation of Candidate Gene Clusters Underlying Competitive Ability in *Sinorhizobium meliloti* using CRISPR-Cas3

Olayemi Bakare

Abstract

Biological nitrogen fixation through rhizobia-legume symbiosis provides an environmentally friendly alternative to chemical fertilizers and plays a substantial role in sustainable agriculture. To enhance this process, farmers often apply rhizobial inoculants to improve nodulation and nitrogen input. However, these plant-beneficial microbial inoculants often fail in the field because less effective native soil microbes outcompete them for nodule occupancy, reducing benefit to the plant partner. Although this rhizobia competition problem is widely described as a barrier to inoculant performance, yet, the bacterial genetic determinants remain largely uncharacterized, limiting strain improvement. To address this gap, we assembled the genomes of 185 *S. meliloti* strains and performed a PanGWAS analysis with Scoary2, using relative abundance as the competitive phenotype. From this analysis, we selected six candidate gene clusters distributed between the chromosome and the PsymB megaplasmid for association with competitiveness. To test whether these associations reflect determinants of competitive ability, we designed guide RNAs for CRISPR-Cas3 targeted deletions in *S. meliloti* KH46C and verified successful mutants by whole-genome sequencing. Each validated deletion mutant will be tagged with a plasmid identifier and evaluated in competition assays against the wild-type parent in alfalfa. Any candidate with impaired competitiveness or that exhibits a nodule occupancy defect will provide evidence for this important trait, allowing us to pinpoint loci that influence rhizobial competitive ability. Overall, this work helps reveal genetic factors that influence competitiveness, contributing to a better understanding of traits relevant to inoculant success in real agricultural soils.

P-54. Characterizing the role of *phyR* gene in formaldehyde homeostasis and methylotrophic adaptation in *Methylobacterium extorquens* PA1

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Abstract

Microbial life in the phyllosphere requires constant adaptation to nutrient scarcity and fluctuating environmental stresses. The facultative methylotroph *Methylobacterium extorquens* uses plant-derived methanol as a carbon and energy source, producing the toxic compound formaldehyde (FA) as an essential intermediate during its oxidation. During a shift to methanol dependent growth, methanol oxidation can outpace FA processing, allowing toxic FA to accumulate. Two FA-responsive proteins, the sensor EfgA and transcription factor TtmR, help maintain FA balance during this transition; loss of either increases resistance to exogenous FA but impairs the transition to methylotrophic growth. PhyR, a general stress response regulator in Alphaproteobacteria, helps *M. extorquens* withstand phyllosphere stresses, including desiccation, oxidative stress, and osmotic stress. PhyR uses a partner-switching mechanism involving the antisigma factor NepR and alternative sigma factor EcfG. Despite its established role in plant colonization, the role of PhyR in methylotrophic metabolism remains poorly understood. Here, we investigated how PhyR contributes to FA homeostasis and transitions to methylotrophic growth in the *Arabidopsis* isolate PA1. Deletion of *phyR* prolonged the lag phase during transitions from multi-carbon substrates to methanol. During the succinate-to-methanol transition, Δ*phyR* accumulated approximately 900 μM extracellular FA, 14.5-fold higher than wild type and 3.0-fold higher than either Δ*efgA* or Δ*ttmR* mutants. This is consistent with disrupted FA homeostasis during the shift to methylotrophy. The pronounced extracellular FA accumulation coincided with an initial decline in Δ*phyR* mutant viability, followed by population recovery. Together, these results indicate that loss of PhyR disrupts FA balance and compromises survival during the transition to methanol-dependent growth. Like Δ*efgA* and Δ*ttmR*, Δ*phyR* also showed increased resistance to exogenous FA despite its impaired transition to methylotrophic growth. Deleting *phyR* in either mutant background further exacerbated methylotrophic transition defects, suggesting that PhyR makes a distinct contribution alongside EfgA and TtmR. Together, these findings reveal a tradeoff between resistance to exogenous FA and efficient adaptation to methanol. These results are consistent with a role for PhyR in maintaining FA balance during metabolic transitions and suggest a connection between general stress regulation and metabolic flexibility in fluctuating plant-associated environments.

P-55. Engineering Oxygen-Resilient Nitrogen Fixation Inspired by Thermophilic Cyanobacteria from Yellowstone

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Abstract

Biological nitrogen fixation is important for sustainable agriculture and biomanufacturing, but nitrogenase's oxygen sensitivity limits activity in oxygenated environments and complicates its use in engineered systems. How can nitrogen fixation be sustained where oxygen production, environmental stress, and nutrient limitation coexist? Thermophilic cyanobacteria offer a promising but understudied system for addressing this question. Filamentous forms can separate oxygenic photosynthesis from nitrogen fixation through cellular differentiation,

while their adaptation to heat and fluctuating geochemistry may involve stress-tolerance mechanisms that remain poorly understood. To interrogate this opportunity, we investigated cyanobacterial communities from four Yellowstone geothermal springs: Mushroom Pool, Five Sisters Spring, White Creek, and Octopus Spring and used IC, ICP-MS, and TOC/TIC to characterize their nutrient poor, ion rich, inorganic carbon dominated conditions. Selective enrichments in nitrogen depleted media at 45°C and 55°C suppressed mesophilic competitors, and secondary recovery by agar plating and filter-pad isolation yielded stable filamentous cultures. Whole genome nanopore sequencing identified *Fisherella thermalis* (formerly known as *Mastigocladus*) at 45°C and *Neowestiellopsis persica* at 55°C. The next step will be to validate nitrogen fixation activity through quantitative assays, alongside metagenomic analysis of the original environmental samples to capture full community diversity, identify nitrogen fixation related genes, and contextualize the isolates within their native environments. Organisms that naturally sustain this fragile metabolism under sustained geothermal stress may reveal transferable strategies for oxygen management, thermal tolerance, and stable nitrogenase function, informing the design of high temperature phototrophic reactors, distributed nitrogen generation platforms, and more durable biological solutions for sustainable nutrient cycling and next generation bioprocess design.

P-56. Discovering New Antibacterials Through AI-Assisted Screening and Experimental Validation

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Abstract

The growing spread of antibiotic-resistant bacteria is a major global health concern that drives the search for new antibacterial strategies and molecular targets. Conventional antibiotics primarily target active cellular processes, and their effectiveness can decrease against non-dividing bacterial populations, including antibiotic-tolerant persister cells, which survive antibiotic exposure without heritable resistance while remaining non-dividing. These surviving populations can contribute to recurrent infections. Our approach is to target cellular components, such as proteases, that remain functional during different bacterial growth states. ClpP is a highly conserved bacterial protease involved in protein quality control and degradation. ADEP4 is a peptide antibiotic that binds ClpP, opens its proteolytic chamber, and causes uncontrolled protein degradation, leading to bacterial cell death. Inspired by this approach, we conducted *in silico* screening of small molecules using molecular docking, structural chemistry, and pharmacokinetic profiling, guided by the known ADEP4-binding region of ClpP. Our initial efforts identified 2 structurally distinct compounds, AGI-6780 and Birabresib, that had not previously been reported as antibacterials. Both exhibited antimicrobial activity against several Gram-positive bacterial species. Here, we expanded the discovery pipeline through larger computational screens, AI-assisted approaches, and additional protease targets. Of 40 candidates selected for experimental testing, 7 exhibited antimicrobial activity against multiple bacterial species, including clinically relevant and ESKAPE pathogens. None had prior reports of antimicrobial activity. Ongoing studies are evaluating the potency, spectrum of activity, toxicity, and mechanism of action of these compounds.

P-57. Molecular Cloning and Characterization of Biopolymer-Synthesizing Enzymes from *Methylocystis suflitae*

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Abstract

Methanotrophic bacteria offer a sustainable route for converting methane into value-added biodegradable polymers. Previous work with *Methylocystis suflitae* demonstrated efficient polyhydroxyalkanoate production and identified four distinct paralogous genes encoding storage-inclusion synthesis enzymes. Computational modeling and substrate-docking analyses further suggested substantial structural diversity among these enzymes and differences in their interactions with hydroxyalkanoate substrates, indicating that individual enzyme variants may contribute differently to polymer composition (Moutsoglou et al. 2024). Building directly on those findings, the present study focuses on the molecular cloning and experimental characterization of these four genes to establish their structure–function relationships. Genomic DNA from *M. suflitae* is being used as the template for amplification of each target coding sequence, followed by insertion into suitable expression vectors and transformation into a heterologous host for recombinant protein production. Clones will be screened and sequence-verified prior to protein expression and purification. Purified recombinant enzymes will then be subjected to X-ray crystallography to resolve their three-dimensional structures and compare catalytic regions, substrate-binding pockets, and other structural features that may govern substrate specificity. In parallel, the individual enzyme variants will be evaluated for their ability to generate distinct polyhydroxyalkanoate compositions, enabling direct correlation between enzyme structure and the resulting biopolymer profile. This work will experimentally validate predictions from the earlier genomic and computational study and determine how paralog-specific structural differences influence biopolymer diversity. Ultimately, these findings will provide a molecular foundation for engineering methane-derived polyhydroxyalkanoates with tailored compositions, thermal behavior, and material properties for sustainable bioplastic applications and broaden opportunities for development of commercially relevant biodegradable materials.

P-58. Genome-Wide Prediction of Non-Coding and Small RNAs in *Aspergillus* and Their Regulatory Link to Aflatoxin Biosynthesis

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Abstract

Filamentous fungi, including *Aspergillus parasiticus* and *A. flavus*, produce carcinogenic aflatoxins that contaminate susceptible crops such as peanuts, maize, cotton, and tree nuts. Despite >90% sequence similarity across their eight homologous chromosomes, *A. parasiticus* SU-1 produces both B- and G-type aflatoxins at high levels, whereas *A. flavus* predominantly produces B-type aflatoxins. Understanding regulatory mechanisms underlying these differences is essential for developing strategies to mitigate aflatoxin contamination.
Here, we characterized the genome-wide non-coding transcriptome (Regulome) of *A. parasiticus* SU-1 to identify potential post-transcriptional regulators of development, stress adaptation, and secondary metabolism, in particular aflatoxin biosynthesis. ncRNA sequences were predicted and annotated against Rfam models, yielding **38 unique functional ncRNA and sRNA families**. Among these were specialized and previously uncharacterized *Aspergillus* sRNAs, including the bZIP Hac1/Xbp1 intron sRNA (RF04247), 5′ *ureB* sRNA (RF02514), and three uncharacterized families: Afu_182 (RF01496), Afu_254 (RF01504), and Afu_309 (RF01512). These ncRNAs represent candidate regulatory nodes linking cellular physiology to aflatoxin biosynthesis. The bZIP Hac1/Xbp1 intron sRNA is associated with non-

canonical splicing during the unfolded protein response, a pathway that can influence secretion and secondary metabolism. The 5′ *ureB* sRNA may connect nitrogen utilization with metabolic regulation, potentially affecting aflatoxin biosynthetic activity. Afu_182, Afu_254, and Afu_309 may represent novel regulatory sRNAs involved in transcript-specific or RNAi-associated regulation. This genome-wide ncRNA catalog provides a foundation for experimentally validating regulatory networks controlling aflatoxin biosynthesis and identifying molecular targets for reducing aflatoxin contamination.

P-59. Comparative Genomics of *Listeria monocytogenes* from Ruminant Neurolisteriosis Cases Reveals Novel Plasmids with Potential Virulence Genes

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Abstract

Listeria (*L.*) *monocytogenes* is a foodborne pathogen that causes listeriosis in humans. Although relatively rare compared to other food-borne diseases, human listeriosis is characterized by a high mortality rate. L. monocytogenes is also found in the agricultural environment and is known to cause disease in ruminants. Similar to humans, severe cases of ruminant listeriosis can be neuroinvasive, leading to encephalitis and meningitis. In this study, seven neuroinvasive *L. monocytogenes* isolates were obtained from listeriosis cases in sheep, goats, and cattle. Genome sequencing was performed using Oxford Nanopore Technologies to characterize these isolates and their virulence potential. Two of the isolates belonged to multilocus-sequence type (MLST) ST1, a clade of hypervirulent *L. monocytogenes* strains. Genome analysis revealed that all isolates harbored key virulence genes. Two isolates (26-140 and 26395) contained novel plasmids encoding genes that may confer increased virulence. These genes include predicted surface proteins or adhesins and putative toxins. We were unable to remove the plasmids using novobiocin and heat, indicating that these plasmids are essential. Listeria plasmids contribute to stress response under various conditions; the presence of two plasmids that are only distantly related to known *Listeria* plasmids and are predicted to contribute to virulence is a novel finding. Future research is required to functionally characterize these plasmids and their contribution to virulence. This will include performing Reverse-Transcriptase quantitative PCR (RT-qPCR) assays to study the gene expression of the plasmid-encoded virulence genes in a cell culture system and the cloning and expression of the genes in *Listeria monocytogenes*.

P-60. A defined gastrointestinal bacterial inoculum for gnotobiotic severe combined immunodeficient pigs: Investigating stability and safety over time

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Abstract

There is a critical need to develop a porcine animal model with a humanized immune system to advance biomedical research. Porcine models are valuable for research translating to human medicine due to similarities in physiology and immune capacity. However, much work, including testing human tissue transplants, cannot be performed in pigs, as the tissue would be rejected. Pigs with severe combined immunodeficiency (SCID), which lack an adaptive immune system, can be utilized to fill the need for this model. SCID pigs are raised in a strict biocontainment facility but are highly susceptible to infection. To provide protection against

pathogens and encourage gastrointestinal tract (GIT) development, we are developing a defined bacterial inoculum to establish a beneficial GIT microbiome. The objective of this study was to investigate the stability and safety of the SCID pig inoculum. Seven porcine GIT bacterial strains were administered via oral gavage on day zero and one to a starting population of 25 SCID and 10 wild-type pigs delivered by cesarean section. Fecal samples were collected between day zero through four weeks of age for 16S rRNA amplicon sequencing, revealing detectable levels of all seven inoculum strains through three weeks of age. Whole-genome sequencing of strains comprising the SCID inoculum was performed to investigate their genetic potential and revealed no genes associated with virulence and antimicrobial resistance. While additional data are needed to make conclusions, these results are encouraging regarding the inoculum's stability and safety for further development and refinement of the SCID pig animal model.

P-61. Mitigation of cell death pathways to combat porcine reproductive and respiratory syndrome virus pathogenesis

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Abstract

Apoptosis is a major form of regulated cell death regulated by the family of cysteine proteases termed caspases. Apoptotic cell death has been associated with PRRSV infections, while the roles of other lytic forms of regulated cell death, such as necroptosis and pyroptosis, and their roles in governing membrane damage have remained poorly understood. In this study, we investigated the mechanisms of PRRSV-induced cell death and evaluated the role of membrane repair pathways in the mitigation of membrane damage during PRRSV infection. Cell death was quantified using flow cytometry, validated by Western Blot analysis of apoptotic markers. PARP cleavage shows the activation of apoptosis during PRRSV induced cell death. When treated with zVAD, a broad spectrum caspase inhibitor, cell death was not significantly reduced, indicating that PRRSV-induced cytotoxicity is not exclusively apoptotic. When treated with NSA, a membrane pore inhibitor, and zVAD in combination, the cell death was reduced. Thus, combined inhibition of apoptosis and non-apoptotic cell death associated with membrane pore formation totally blocked PRRSV infection induced cell death. Furthermore, using butyrate to enhance membrane repair and stability led to increased cell survival in PRRSV infected cells. These findings demonstrate that PRRSV-induced cell death also involves caspase-independent lytic mechanisms driven by membrane damage. Enhancing membrane repair may therefore represent a promising therapeutic strategy to limit tissue damage and improve host survival during PRRSV infection.

P-62. Investigating the Role of LMO2269 in *Listeria monocytogenes* stress response

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Abstract

Listeria monocytogenes is a Gram-positive foodborne pathogen known for its ability to withstand various stressors associated with harsh conditions in both the environment and food production facilities. The stress response mechanisms of *L. monocytogenes* are relatively well studied; however, many genes within these systems remain functionally uncharacterized. One such gene is *lmo2269*, an uncharacterized gene regulated by the alternative sigma factor σ^B, the primary stress regulator in *L. monocytogenes*. *Lmo2269* has been highly upregulated in

response to a variety of food processing environment (FPE) related stressors shown in previous studies. To investigate the role of *lmo2269* in stress response, we generated a deletion mutant of *lmo2269* in *L. monocytogenes* strain 6179. A series of growth curves and survival assays were performed to characterize the role of *lmo2269* during exposure to stress conditions relevant to FPEs such as acid, osmotic, and oxidative stress. From these results, it is suggested that *lmo2269* plays some role in osmotic stress response, although this function may be growth phase, osmolyte concentration, or exposure-time dependent. Additionally, complementation of the deletion mutant revealed that constitutive expression of *lmo2269* significantly hinders the growth of the cell. Finally, future work is proposed and will be necessary to fully understand the role of *lmo2269* in *L. monocytogenes* stress response and survival.

P-63. Development and Validation of a Primary Embryonic Chicken Tracheal Cell Model for the Characterization of U.S. aMPV Subgroups

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Abstract

The simultaneous emergence of avian metapneumovirus (aMPV) subtypes A and B in the United States poultry industry has created a critical need for standardized, host-specific diagnostic platforms. Historically, reliance on continuous, multi species cell lines has limited the primary isolation of field strains and introduced potential adaptation artifacts. This study addresses these challenges by utilizing a robust, primary chicken-cell system to directly isolate, adapt, and characterize the comparative dynamics of U.S. aMPV-A and aMPV-B. In this study we first established primary embryonic chicken tracheal (ECT) cultures and phenotyped them using various cellular markers. Primary ECT cell monolayers were inoculated with aMPV viral strains to assess host susceptibility, replication kinetics, and protein expression. Using an optimized four-day harvest protocol, viral infections were confirmed by real-time Reverse Transcription-Polymerase Chain-Reaction (qRT-PCR) and visualized via indirect immunofluorescence assay (IFA), utilizing a monoclonal mouse anti-aMPV-B nucleoprotein (NP) primary antibody and FITC-conjugated secondary antibody. Results demonstrate that this species-specific primary system effectively supports the viral entry, replication, and protein synthesis of both subtypes, producing distinct, strain-specific fluorescence patterns. Specifically, aMPV-A exhibited intense, punctate branching networks, while aMPV-B displayed heavily granular focal clusters. These findings validate the primary chicken-cell model as a biologically relevant platform that may avoid the mutations and viral adaptations associated with continuous cell lines. This standardized approach provides a consistent substrate for comparative virological research, facilitating more accurate studies of viral pathogenesis, vaccine candidate evaluation, and diagnostic optimization for emerging aMPV strains in the U.S. poultry sector.

P-64. Impact of Bacterial and Viral Ligands on Intestinal Barrier Function in Polarized Porcine Epithelial Cells

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Abstract

Intestinal epithelial cells play a significant role in the pathogenesis of enteric diseases and in mediating innate immune responses in the gastrointestinal tract. Tight junctions (TJs) are essential for maintaining epithelial barrier integrity by regulating paracellular permeability and

preventing the translocation of harmful substances and microorganisms. Disruption of TJs leads to increased intestinal permeability, commonly referred to as leaky gut, which is associated with several gastrointestinal disorders. Bacterial and viral ligands, such as flagellin, polyinosinic: polycytidylic acid (poly I:C), and imiquimod, can modulate epithelial barrier function, potentially through Toll-like receptor (TLR) mediated signaling pathways. However, their effects on intestinal epithelial barrier integrity remain poorly understood. In this study, we developed an in vitro polarized porcine intestinal epithelial model using IPEC-J2 cells to investigate the dose and time-dependent effects of these ligands on epithelial barrier function. Barrier integrity was assessed by measuring transepithelial electrical resistance (TEER), and immunofluorescence staining was used to evaluate the localization of key tight junction proteins, including ZO-1, claudin-1, claudin-3, and occludin. Treatment with flagellin, poly I:C, and imiquimod resulted in significant dose and time dependent reductions in TEER, indicating compromised epithelial barrier integrity. These findings provide mechanistic insights into how microbial ligands influence intestinal permeability and contribute to epithelial barrier dysfunction. Furthermore, this in vitro model may serve as a useful platform for evaluating therapeutic strategies aimed at restoring epithelial barrier function and preventing gastrointestinal disease progression.

P-65. Implementing SynBio-CURE: A Flexible Course-Based Undergraduate Research Experience in Microbiology and Synthetic Biology

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Abstract

Course-based undergraduate research experiences (CUREs) engage students in authentic research while developing laboratory and scientific reasoning skills. We recently implemented SynBio-CURE, an NSF-supported research-intensive course designed to move students from foundational training toward independent research. Rather than assigning all students to a single research project, students work on individual or collaborative projects spanning antimicrobial discovery, including AI-assisted approaches to antibiotic discovery, bacterial survival during antibiotic exposure, protein purification and characterization, microbiome analysis, and synthetic biology research. We developed a structured skills-progression framework to build technical competence, critical thinking, scientific curiosity, and confidence while maintaining safety and oversight. Students receive core training in laboratory techniques, equipment use, and primary literature analysis through dry-run and live-culture activities, competency checks, self-assessments, and instructor signoffs. As students demonstrate proficiency, they gain increasing independence. This flexible progression allows students with different backgrounds and projects to advance at appropriate rates. A defining feature of the course is that students investigate authentic research questions with unknown outcomes rather than traditional laboratory exercises with predetermined results. This open-ended environment has produced strong student engagement as students develop skills in experimental design, troubleshooting, protocol adaptation, interpretation of unexpected results, and scientific decision-making. These skills are challenging to develop through traditional laboratory coursework alone. Student reflections and competency assessments will be used to evaluate changes in confidence, critical thinking, scientific curiosity, and research independence. SynBio-CURE provides a scalable framework for supporting diverse authentic research projects within an undergraduate course while promoting scientific inquiry, collaboration, and ownership of the research process.