POSTER ON BOARDS

SHIFT 02

SHIFT 02-001

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

TARGETED THERAPY AGAINST SHIGELLA: ANTIBODY-DRUG CONJUGATES (ADCS)

Lecture Title:

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Background and Aims: *Shigella* is a Gram-negative bacterium belonging to the Enterobacteriaceae family and represents the leading cause of diarrheal infections worldwide. It prevalently affects children in LMIC, resulting in more than 160,000 deaths annually, highlighting the need for effective prevention and treatment strategies. ADCs typically combine a monoclonal antibody (mAb) and toxic payloads via a chemical linker, and they could offer a promising alternative to conventional antibiotics. Recent developments of an antibody-antibiotic conjugate (AAC) against *Staphylococcus aureus* demonstrated the safety and tolerability of a compound capable of efficiently eliminating bacteria *in vitro* and *in vivo*. These advancements in AAC research offer a promising avenue that could help addressing the challenges posed by *Shigella* infections

Methods: Our team have successfully established a high-throughput pipeline to isolate human monoclonal antibodies from individuals exposed to *Shigella* infections. Our lead candidate delivers both opsonophagocytic and bactericidal activity. This project is aimed at generating AACs to further improve the therapeutic efficacy of our lead mAb candidate.

Results: The initial proof of concept for the establishment of the chemical-conjugation protocol was performed with doxorubicin. Successful conjugation and drug to antibody ratio was assessed by mass spectrometry and HPLC. Further characterization included aggregation propensity and thermal stability by size-exclusion chromatography and differential scanning fluorimetry respectively. We are currently exploring the ability of this ADC in improving opsonophagocytic killing of *S. sonnei*.

Conclusions: These findings will guide in the selection of the best assays and biochemical properties for the generation of a novel AAC against *S. sonnei*.

Disclosure: No significant relationships.

Keywords: Antibody Drug Conjugates, Shigellosis, Antibody Antibiotic Conjugates, Monoclonal antibodies, Shigella sonnei

SHIFT 02-002

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

ANTIMICROBIAL STEWARDSHIP IN AN EMERGENCY GENERAL SURGERY SETTING: IMPROVING RATES OF ADHERENCE TO LOCAL INTRAOPERATIVE ANTIBIOTIC GUIDELINES FOR GENERAL SURGICAL PROCEDURES

Lecture Title:

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Background and Aims: Intraoperative antibiotic prophylaxis has been proven to minimise the risk of surgical site infections for specific procedures. However, this must be balanced against adverse effects of antibiotics and the risk of spreading antibiotic resistance by consistently adhering to local formularies wherever possible. This Quality Improvement Project aimed to audit the rates of adherence to local intraoperative antibiotic guidelines within the Emergency General Surgery setting at a single centre in West London, UK, and assess improvements following intervention via a follow-up audit cycle.

Methods: The "Cerner" Electronic Health Record was used to retrospectively identify 50 patients under the Emergency General Surgery setting who had undergone surgical procedures requiring intraoperative antibiotic prophylaxis according to the local hospital guidelines between 01/11/23 - 23/12/23; antibiotics administered were compared to local guidelines. Following discussions with members of the surgical team, an informational poster displaying common indications and concisely laid out guidelines was formulated and displayed both physically in surgical offices and online via the local surgical training forum. A second audit cycle was then performed between 17/01/24 - 14/02/24.

Results: The initial audit cycle showed a 30% adherence rate to intraoperative antibiotic guidelines; common reasons included previous hospitals having different guidelines and unfamiliarity with exact indications for antibiotic prophylaxis. Following discussions with surgeons and dissemination of the informational poster, the adherence rate increased to 57%.

Conclusions: Adherence to local formularies when prescribing intraoperative antibiotics are important for reducing spread of antibiotic resistance; suboptimal adherence rates can evidently be rapidly improved via simply making guidelines more visible and accessible.

Disclosure: No significant relationships.

Keywords: Intraoperative, Antibiotic, Stewardship, General Surgery, Guidelines

SHIFT 02-003

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

RECURRENT SALMONELLA ENTERICA SUBSP. ENTERICA SEROVAR ENTERITIDIS BACTEREMIA AND ANTIBIOTIC RESISTANCE IN SINGLE MEDICAL CENTER IN TAIWAN

Lecture Title:

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Background and Aims: *Salmonella*, a genus of Gram-negative bacteria belonging to the family *Enterobacteriaceae*, comprises two main species: *Salmonella enterica* and *Salmonella bongori*. In our investigation, we focus on analyzing local cases of bloodstream infection caused by *Salmonella Enteritidis*, particularly those characterized by persistence or recurrence. Clinical characteristics and antibiotic resistance are emphasized in our study.

Methods: This study examined 12 cases of persistent or recurrent bloodstream infections attributed to *Salmonella Enteritidis* at Taichung Veterans General Hospital. The investigation involved review of patients' electronic medical records and the documentation of minimum inhibitory concentrations (MICs) for antibiotics across 25 *Salmonella* samples. Additionally, the samples underwent whole genome sequencing as part of the analysis.

Results: From 2015 to 2018, 25 blood culture samples from these patients were examined. Comprehensive details on clinical conditions and additional positive culture outcomes outline in Table
1.

Table 1 Clinical characteristics among 12 patients

Patient	Age	Gender	Stool Culture	Other Culture	Immunocompriosed status	Mycortic Aneurysm	SIRS Crteria*	Known risk factor
P1	73	F	+	Urine	Acute Myeloid Leukemia		3, 3	
P2	75	F			Breast Cancer		3, 3	
P3	93	M				+	3, 3	
P4	68	M					3, 4	Uncooked food
P5	67	M			Cancer of unknown origin		2, 3	
P6	74	M		Urine	Hepatocellular carcinoma		3, 3	
P7	77	F				+	3, 3	
P8	16	F			Ambiguous leukemia		4, 4	
P9	69	M		Ascites	Hepatocellular carcinoma		2, 4	
P10	52	M		Urine	Mutiple Myeloma		3, 3, 4	
P11	39	M	+		Human Immunodeficiency Virus		3, 2	
P12	44	M			Human Immunodeficiency Virus Diffuse large B cell lymphomas		2, 3	

*SIRS Criteria : Body temperature over 38 or under 36 degrees Celsius, Heart rate greater than 90 beats/minute, Respiratory rate greater than 20 breaths/minute or partial pressure of CO2 less than 32 mmHg, Leukocyte count greater than 12000 or less than 4000 /microliters or over 10% immature forms or bands.

As for the minimum inhibitory concentration (MIC) for antibiotics across the 25 samples, detailed findings are listed in Table 2. The allelic profiles of seven housekeeping genes (aroC, dnaN, hemD, hisD, purE, sucA, and thrA) are provided in Table 3, There is no discernible differences among them, and all sequences type is ST11. It is possible that antibiotic resistance may be linked to allelic profiles beyond those mentioned
above.

Table 2 MIC(minimum inhibitory concentration) of antibiotic in each sample

Sample	SAM	SXT	CRO	CIP	TZP	IMP
1-1	R>32	S<=20	S<=1	S<=0.25	S<=4	S<=0.25
1-2	S<=2	S<=20	S<=0.25	S<=0.25	S<=4	S<=0.25
2-1	S<=2	S<=20	S<=0.25	S<=0.25	S<=4	S<=0.25
2-2	S<=2	S<=20	S<=0.25	S<=0.25	S<=4	S<=0.25
3-1	S<=2	S<=20	S<=1	S<=0.25	S<=4	S<=0.25
3-2	S<=2	S<=20	S<=1	S<=0.25	S<=4	S<=0.25
4-1	S<=2	S<=20	S<=1	I=0.5	S<=4	S<=0.25
4-2	S<=2	S<=20	S<=1	S<=0.25	S<=4	S<=0.25
5-1	S<=2	S<=20	S<=0.25	S<=0.25	S<=4	S<=0.25
5-2	S<=2	S<=20	S<=0.25	S<=0.25	S<=4	S<=0.25
6-1	S<=2	S<=20	S<=0.25	S<=0.25	S<=4	S<=0.25
6-2	S<=2	S<=20	S<=0.25	S<=0.25	S<=4	S<=0.25
7-1	S<=2	S<=20	S<=0.25	S<=0.25	S<=4	S<=0.25
7-2	S<=2	S<=20	S<=0.25	S<=0.25	S<=4	S<=0.25
8-1	R>32	R>320	S<=0.25	S<=0.25	S<=4	S<=0.25
8-2	R>32	R>320	S<=0.25	S<=0.25	S<=4	S<=0.25
9-1	S<=2	S<=20	S<=1	S<=0.25	S<=4	S<=0.25
9-2	S<=2	S<=20	S<=1	S<=0.25	S<=4	S<=0.25
10-1	R>32	S<=20	S<=0.25	S<=0.25	S<=8	S<=0.25
10-2	R>32	S<=20	S<=0.25	S<=0.25	S<=16	S<=0.25
10-3	R>32	S<=20	S<=0.25	S<=0.25	S<=16	S<=0.25
11-1	R>32	R>320	S<=0.25	S<=0.25	S<=4	S<=0.25
11-2	R>32	R>320	S<=0.25	S<=0.25	S<=4	S<=0.25
12-1	R>=32	S<=20	S<=1	S<=0.25	S<=4	S<=0.25
12-2	R>=32	S<=20	S<=1	S<=0.25	S<=4	S<=0.25

SAM: Ampicillin/Sulbactam, SXT: Trimethoprim/Sulfamethoxazole, CRO: Ceftriaxone, CIP: Ciprofloxacin, TZP: Piperacillin/Tazobactam, IMP: Imipenem

Table 3 allelic profiles of each sample

Sample	aroC	dnaN	hemD	hisD	purE	sucA	thrA
1-1	5	2	3	7	6	6	11
1-2	5	2	3	7	6	6	11
2-1	5	2	3	7	6	6	11
2-2	5	2	3	7	6	6	11
3-1	5	2	3	7	6	6	11
3-2	5	2	3	7	6	6	11
4-1	5	2	3	7	6	6	11
4-2	5	2	3	7	6	6	11
5-1	5	2	3	7	6	6	11
5-2	5	2	3	7	6	6	11
6-1	5	2	3	7	6	6	11
6-2	5	2	3	7	6	6	11
7-1	5	2	3	7	6	6	11
7-2	5	2	3	7	6	6	11
8-1	5	2	3	7	6	6	11
8-2	5	2	3	7	6	6	11
9-1	5	2	3	7	6	6	11
9-2	5	2	3	7	6	6	11
10-1	5	2	3	7	6	6	11
10-2	5	2	3	7	6	6	11
10-3	5	2	3	7	6	6	11
11-1	5	2	3	7	6	6	11
11-2	5	2	3	7	6	6	11
12-1	5	2	3	7	6	6	11
12-2	5	2	3	7	6	6	11

Conclusions: Our study focused on patients with recurrent *Salmonella Enteritidis* bacteremia. Despite analyzing seven housekeeping genes (aroC, dnaN, hemD, hisD, purE, sucA, and thrA), no significant differences were noted.Therefore, further investigations into other alleles and epidemiology may be investigated in future.

Disclosure: No significant relationships.

Keywords: Salmonella, Enteritidis, Antibiotic resistance, Taiwan, bacteremia

SHIFT 02-004

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

ANALYSIS OF DISTRIBUTION AND DETECTION OF EXTENDED SPECTRUM BETA-LACTAMASES/CARBAPENEMASES/OXACILLINASES: AN 10-YEAR RETROSPECTIVE STUDY, WHERE ARE WE TODAY IN CONCEPT "ONE HEALTH"

Lecture Title:

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Background and Aims: Among the global health problems, antimicrobial-resistance (AMR) is the one that most clearly illustrates the One Health approach.

Methods: Double-disk-synergy test was used to screen for ESBLs. PCR was used to detect bla_{ESBL} alleles.

Results: In 2010, among 85 UTIs caused by ESBL-producing Gram-negative bacteria, 44 (51.8%) were obtained from in- and 41 (48.2%) from outpatients. Twentyone (75.0%) in- and nine (47.4%) outpatients *Klebsiella* spp. were positive for bla_{TEM}, whereas 27 (96.4%) and 6 (31.6%) were positive for bla_{CTX-M}, (18 in-patient isolates were produced $bla_{CTX-M-15}$ and two $bla_{CTX-M-3}$). In 2013, among environmental samples, 52 (out of 381 Gram-negative bacteria, 13.6%) were ESBL-producing isolates: 37 (71.2%) from water, seven (13.5%) from food and eight (15.4%) from environmental surfaces. The most prevalent ESBL-producing bacteria isolated from environmental samples was *E. coli* 26 (50.0%), *Klebsiella* spp. 10 (19.2%), non-fermentors 9 (17.3%) and other bacteria from seven (13.5%) samples. In 2016, seven *Acinetobacter baumannii* isolates with reduced susceptibility to carbapenemas were. All strains were found to be positive for $bla_{OXA-24/40}$. In 2019, Among 108 swabs collected from poultry feces, 75 (69.4%) were positive on *E. coli*, of which 27 (36.0%) were cefotaxime-resistant. Fourteen isolates yeielded amplicons with primers specific for TEM. Multiplex PCR revealed group 1 (21 out of 27), $bla_{CTX-M-15}$ (seven out of 27) of CTX-M beta-lactamases.

Conclusions: Our Country is one of the rare countries in EU, who does not have National Action Plan on Antimicrobial Resistance, and we must create a plan to combat this honor as soon as possible.

Disclosure: No significant relationships.

Keywords: Antimicrobials, Human, Animals, One Health

SHIFT 02-005

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

THE PREVALENCE OF NOSOCOMIAL INFECTION IN A CANTONAL HOSPITAL ZENICA, BOSNIA AND HERZEGOVINA

Lecture Title:

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Background and Aims: Nosocomial infection (NI) has always been considered a significant problem around the world.

Methods: This was a cross-sectional study of patients admitted to Hospital, from January 2022 through December 2022. Pathogens were identified using standard microbiological methods, and antimicrobial susceptibility was determined by disk diffusion tests according to the Clinical and Laboratory Standards Institute guidelines.

Results: A total of 24.968 patients were admitted to the hospital during the study period, including 86 found to have NIs (approximately 0,3%). These included 56 male (65.1%) and 30 female (34.9%) patients. Almost all patients were older than 50 years (94.2%/n=81). The majority (44.2%) of the NIs were reported from the Department for anesthesia, resuscitation and rehabilitation (n=38, out of 86), followed by the Internal medicine with hemodialysis (30.2%, n=26), Department for infectious diseases (12.8%, n=11), Neurology (4.6%, n=4), Orthopedics and Traumatology (3.5%, n=3) and one case in each of the Pediatric/Physical/Chirurgy and Oncology department. The most common site of infection was the gastroenteritis (59.3%, n=51), followed by the pneumonia (22.1%, n=19), bacteremia (7.0%, n=6), bacteriuria and urotract infection (9.3%, n=8) and surgical wards (2.3%, n=2). Among the pathogens isolated, *Clostridium difficile* and *Acinetobacter* spp. were the most common (59.3%, n=51 and 36.0%, n=31) followed by *Serratia* spp. (2.3%, n=2) and one of each *Klebsiella* spp. and *Pseudomonas* spp. Among *Acinetobacter* spp. resistance rates to ciprofloxacin, imipenem, sulpfametoxazol-trimetoprim, ceftazidime, cefepime and amikacin were 96.6%, 93.1%, 93.1%, 89.7%, 89.7% and 89.7%, respectively.

Conclusions: Nosocomial infection is considered an important challenge in the health system.

Disclosure: No significant relationships.

Keywords: Hospital infection, Isolates, Resistance

SHIFT 02-006

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

TIME KILL EXPERIMENTS OF DOUBLE CARBAPENEMS ON CARBAPENEMASE PRODUCING KLEBSIELLA PNEUMONIAE

Lecture Title:

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Background and Aims: Infections with carbapenemase producing enterobacterales (CPE) present a therapeutic challenge. Double carbapenems administered sequentially has been proposed as a potential treatment option. The hypothesis is that the initial carbapenem acts as a carbapenemase inhibitor. This study aims to evaluate the possible enhanced killing effect of double carbapenems *in-vitro*, and whether commencing ertapenem before meropenem increases this.

Methods: Twelve *Klebsiella pneumoniae* isolates carrying the OXA-48 gene were included. Broth microdilution MICs of ertapenem and meropenem were performed, using EUCAST methodology. Time-kill experiments were performed over a 6-hour period. The concentrations of ertapenem and meropenem were adjusted hourly according to pharmacokinetic data (1 gram ertapenem infused over 1 hour, and 2 grams meropenem over 3 hours). Two dosing regimens were applied: ertapenem commencing an hour ahead of meropenem, and both antibiotics commencing simultaneously. The killing effect was compared with meropenem alone, being the carbapenem with the lowest MIC.

Results: The median MIC was 32 mg/L for both carbapenems, (ertapenem range: 16-128 mg/L and meropenem range: 8-64 mg/L). Throughout exposure time the CFU/mL of isolates decreased between 1.5 and 4 logs when ertapenem was used ahead of meropenem, and between 1.5 and 5 logs when both drugs were initiated together. This difference was not statistically significant ($p=0.3$). All isolates showed > 2 log difference with kill using double carbapenems as compared to meropenem alone, indicating synergism.

Conclusions: Carbapenem concentrations comparable with in-*vivo* pharmacokinetics, showed double carbapenems to be more effective than meropenem alone. Administering both drugs simultaneously was as effective as the more demanding sequential use.

Disclosure: No significant relationships.

Keywords: Time kill assay, OXA-48 Klebsiella pneumoniae, Double Carbapenems, Carbapenemase-producing enterobacterales

SHIFT 02-007

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

THE IMPACT OF ANTIBIOTIC PROPHYLAXIS WITH CEFAZOLIN, AND CEFTIZOXIME ALONG WITH AMINOGLYCOSIDES ON POSTOPERATIVE COMPLICATIONS FOR PERCUTANEOUS NEPHROLITHOTOMY

Lecture Title:

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Background and Aims: This study investigated the efficacy of first-generation cephalosporin (cefazolin) and third-generation cephalosporin (ceftizoxime) as prophylactic antibiotics in patients undergoing PCNL surgery. The study also examined the incidence of postoperative complications, hospitalization duration, and return to normal life.

Methods: This prospective cross-sectional study included patients (≥20 years) who underwent PCNL surgery at RAZI, GOLSAR, and PARS Hospitals from January 01, 2013, to December 31, 2022. Patients were divided into two groups and received 1mg/kg of either first-generation (cefazolin) or third-generation (ceftizoxime) intravenously, 30 minutes before the surgery. The incidence of postoperative complications, hospitalization duration, and return to normal life were compared based on the type of prophylactic antibiotic. Risk factors were evaluated using chi-squared tests followed by multivariate logistic regression analysis.

Results: The ceftizoxime group showed significantly lower rates of general complications (13.0% vs 31.4%) and postoperative fever (2.8% vs 15.0%) compared to the cefazolin group. The ceftizoxime group also had a significantly shorter total hospitalization duration (1.31 ± 1.18 days) compared to the cefazolin group (4.03 ± 1.57 days) (p=0.000). Additionally, the ceftizoxime group had a significantly shorter duration for return to normal life (5.97 ± 3.37 days) compared to the cefazolin group (8.15 ± 2.93 days) (p=0.001).

Conclusions: The third-generation prophylactic cephalosporin (ceftizoxime) was superior to the first-generation (cefazolin) in reducing postoperative fever rates, hospitalization duration, and time to return to normal life for patients.

Disclosure: No significant relationships.

Keywords: Cephalosporine, Prophylactic antibiotics, First-generation, Third-generation, Return to normal life

SHIFT 02-008

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

ANTIBIOTIC RESISTANCE PATTERNS OF UROPATHOGENS CAUSING URINARY TRACT INFECTIONS IN CHILDREN UNDER 3 YEARS; A SINGLE CENTER CROSS-SECTIONAL STUDY

Lecture Title:

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Background and Aims: Urinary tract infection (UTI) is one of the most common childhood infections. UTI can lead to serious complications such as hypertension or renal failure, if not diagnosed and treated promptly. Prompt initiation of appropriate empiric therapy in upper UTIs can reduce these complications, which requires the identification of bacteria causing UTIs and their antibiotic resistance patterns.

Methods: 259 children with UTIs from 2014 to 2020 who were hospitalized in 17th Shahrivar Hospital, Rasht, Iran, were included in the study. The Age, sex, clinical symptoms, urine analysis, culture results, and antibiogram of patients were entered into the questionnaire. Then the data were analyzed with SPSS software version 21.

Results: The mean age of the children was 4.9 ± 2.7 months. Boys made up 53.3 percent of the patients. The highest percentage of uropathogenic causing UTIs was Escherichia coli (E. coli), which accounted for 56.4% of all UTIs in children. Then Klebsiella was next with 33.2%. The highest antibiotic susceptibility was observed for ciprofloxacin, nitrofurantoin, amikacin, gentamicin, imipenem, and nalidixic acid. The highest resistance was observed to cephalothin, cephalexin, ampicillin, and amoxicillin.

Conclusions: The most common uropathogenic causing UTIs was E. coli, which is sensitive to ciprofloxacin, amikacin, gentamicin, nitrofurantoin, imipenem, ceftriaxone, and nalidixic acid. Currently, it seems aminoglycosides are drugs of choice in the treatment of UTIs in children under 3 years. In case of any contraindications, third-generation cephalosporins are recommended for empirical treatment and if there is no response to current treatment within 48 to 72 hours, ciprofloxacin can be considered.

Disclosure: No significant relationships.

Keywords: Antibiotic susceptibility, Urinary tract infection, Child health

SHIFT 02-009

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

COULD OXIDATIVE STRESS BE A MODE OF ACTION OF ANTIMYCOBACTERIAL PLANT EXTRACTS?

Lecture Title:

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Background and Aims: Tuberculosis, the most deadly bacterial disease, continues to pose significant epidemiological challenges, particularly with the emergence of Multi-Drug Resistant (MDR) and Extended Drug Resistant (XDR) strains of *Mycobacterium tuberculosis*. The urgency for new antimycobacterial drugs and a deeper understanding of their mode of action is paramount.

Methods: In this work, we tested the antimycobacterial properties of ethanol extracts prepared from aerial-ground parts of 148 plants originating in South America and belonging to 17 different families. The extracts were grouped according to the obtained MIC values for *Mycobacterium tuberculosis* H37Ra. We found two of these with MIC 32 µg/ml, ten MIC 64 µg/ml, fourteen 128 µg/ml, sixty-three 256 µg/ml and 56 above 256 µg/ml. We also determine their ability to generate oxidative stress inside mycobacterial cells with our newly developed technique based on dichlorofloresceín (DCFH) as a ROS detector and 2,2'-azobis (2-amidopropane) dichloride (ABAP) as an artificial ROS generator.

Results: Our results showed that the plant extracts are a valuable source of substances with antimycobacterial properties. We also found that only six extracts could generate oxidative stress comparable to or higher than ABAP-generated positive control.

Conclusions: Our research suggests that the generation of ROS is a relatively rare and rather non-specific mechanism of the antimycobacterial effect of plant extracts.
Acknowledgements*: This work was funded by the Ministry of Education and Science in Poland within the statutory activity of the Medical University of Lublin (DS 5/2023 and DS 28/2023)*

Disclosure: No significant relationships.

Keywords: Tuberculosis, oxidative stress, antimycobacterial activity

SHIFT 02-010

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

ANTIFUNGAL EFFECT OF SELECTED MACROFUNGAL EXTRACTS ON ASPERGILLUS MYCOTOXIGENIC MOULDS

Lecture Title:

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Background and Aims: Pathogenic fungi pose significant threats to both human and animal health, as well as agricultural productivity while mycotoxigenic mould species, especially aflatoxigenic, are dangerous due to the production of the most toxic and harmful mycotoxins. Hence, the main aim of this study was to assess the antifungal activity of 18 different mushroom species, mostly lignicolous, using the microdilution method, against the *Aspergillus* mycotoxigenic moulds: *A. parasiticus, A. flavus* and *A. niger*.

Methods: Fungal extracts dH_2O, 80% ethanol (EtOH), 70% methanol (MeOH) and 5% DMSO concentration (0.09 to 18 mg/mL) were used for detection of their antifungal effect together with *G. pfeifferi* (80% EtOH) on *A. flavus* by the Basilico method, and inhibition of synthesis of aflatoxin by using ultra-high performance liquid chromatography UHPLC-MS/MS.

Results: Significant variations have been detected among mushroom extracts, e.g. *G. applanatum* (5% DMSO) exhibited an antifungal effect (MIC = MFC, 9 mg/mL) on *A. parasiticus* while 80 % EtOH of *G. applanatum*, *G. pfeifferi*, *G. lucidum*, *G. resinaceum* and *T. versicolor* (dH_2O) inhibited *A. flavus* growth (9 mg/mL). Antifungal activity on *A. niger* was demonstrated by seven macrofungal extracts while *S. commune* (5% DMSO) showed lower activity 18 mg/mL, and *G.applanatum (*dH_2O) was active at MIC/MFC 9 mg/mL. Solvents control was negatve so (except 100% DMSO), hence the bioactive compounds from the mushroom are active. In specific, 80% EtOH G. *pfeifferi* reduced synthesis of aflatoxins AFB1 and AFB2 (µg/L), although none antifungal effect was noticed by the method of Basilico.

Conclusions: Mushroom extracts, primarily G*anoderma*, can inhibit the synthesis of aflatoxins and the growth of *Aspergillus* mycotoxigenic molds and should be used in product quality control, but further research is warranted.

Disclosure: Theauthorsgratefully acknowledge the financial support of the Ministry of Science,Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-66/2024-03/ 200125 & 451-03-65/2024-03/200125).

Keywords: antifungal activity, Aspergillus, Ganoderma, moulds, aflatoxins

SHIFT 02-011

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

EVALUATION OF THE ANTIBACTERIAL SUSCEPTIBILITY PATTERN OF CLAY AND SHEA BUTTER COMPOSITES ON SKIN AND WOUND ISOLATES.

Lecture Title:

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Background and Aims: Clays and Shea butter are medicinal materials that have been in existence across ages especially in folk medicines.The aim of the study was to investigate the antibacterial susceptibility pattern of clay leachates,de-fatted Shea butter and different combination of clay and Shea butter composites(1:1, 2:1, 2:3, 3:2)on skin and wound isolates.

Methods: The sensitivity of wound and skin isolates to the clay leachates,de-fatted Shea butter and different combination of clay and Shea butter composite(1:1, 2:1, 2:3, 3:2)were carried out using agar well diffusion method.

Results: The result shown that clay leachate is active against the 100% tested organisms (wound and skin isolates). On wound isolates, *Staphlococcus aureus* had the highest zone of inhibition (20.00±0.00 mm). On skin isolates, *Mycobacterium segmantis* had the highest zone of inhibition (25.00± 5.00mm). De-fatted Shea butter is also active against all tested wound and skin isolates. On wound isolates, the zone of inhibition ranged from 15.00±5.00 mm (*Bacillus cereus)* to 5.00±5.00mm (*Staphylococcus aureus).* On skin isolates, highest zone of inhibition (15.00±5.00mm) *Bacillus cereus* . The clay and Shea butter combination (3:2) and (2:1) had the highest zone of inhibition compared to other ratio on wound isolates. On skin isolates, only combination (3:2) had the highest zone of inhibition compared to other ratio.

Conclusions: In conclusion, clay,Shea butter, clay and Shea butter composites (3:2) possess antibacterial properties which could provide an inexpensive treatment against numerous wound and skin bacterial infections.

Disclosure: No significant relationships.

Keywords: Clay, Shea butter, Clay and Shea butter composites, Antibacterial Susceptibility

SHIFT 02-012

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

MARINE MICROPLASTICS AS VECTORS OF SELECTIVE ENRICHMENT FOR ANTIBIOTIC RESISTANCE GENES

Lecture Title:

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Background and Aims: Microplastic (MP) pollution is a significant issue in marine environments, and MPs can form unique ecological niches on their surface, known as plastisphere. Moreover, MPs were strongly suspicious of the selective enrichment of antibiotic resistance genes (ARGs). However, it is still unclear how different types of MPs can affect the enrichment of ARGs with assemblages of bacterial communities over time in marine environments.

Methods: Four different types of commonly used polymers, namely polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC), were cultured in a marine environment for 46, 63, and 102 days. 16S rRNA gene targeted amplicon sequencing was conducted to identify bacterial communities in the plastisphere. Additionally, the presence of ARGs such as *blaTEM*, *ermB*, *mexF*, *qnrS*, *sul1*, *sul2*, *tetA*, *tetC*, *tetQ*, and *vanA* genes, as well as the *intl1* gene were quantified using qPCR.

Results: The qPCR results showed that the abundance of ARGs continuously increased over 102 days. *TetA*, *tetQ*, *sul1*, and *qnrS* were especially selectively enriched in the PVC-MPs. Additionally, the intl1 gene measured all types of polymers over the cultured periods. Co-occurrence analysis revealed potential hosts for the *ermB*, *tetA*, and *tetQ* genes as *Methylotenera, Coxiella,* and *Pseudahrensia* genera in the plastispheres.

Conclusions: This study showed that the plastisphere can play a critical role in biofilm formation in the field-based marine environment and potentially spread ARGs through horizontal gene transfer.
This work was supported by the National Research Foundation of Korea grant funded by the Korea government(Ministry of Science, ICT & Future Planning[No. 2020R1C1C1008951] and MIST[RS-2023-00219497]).

Disclosure: No significant relationships.

Keywords: Microplastic, Plastisphere, ARGs, Marine environment, Selective pressure

SHIFT 02-013

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

THE ANTIMICROBIAL RESISTANCE PROFILES OF ESHCERICHIA COLI FROM EACH PRODUCTION STAGE OF PIGS IN KOREA

Lecture Title:

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Background and Aims: The use of antimicrobials to treat and prevent disease in pigs is widespread, leading to an increasing likelihood of high levels of antimicrobial resistance (AMR). In this study, we isolated *Escherichia coli* (*E. coli*) from pigs in Korea and investigated the AMR by production stages to analyze its characteristics and trends.

Methods: We isolated 115, 113, 108, 113, and 115 *E. coli* from the feces of the suckling piglets, weaned piglets, grower pigs, finisher pigs and sows respectively. AMR was investigated according to the principles of the Clinical and Laboratory Standard Institute principle.

Results: The resistance to the five antimicrobials was significantly higher in the weaned piglets' feces compared to other production stages; ceftiofur (46.9%), gentamicin (42.5%), kanamycin (63.7%), nalidixic acid (64.6%) and ciprofloxacin (67.3%). Specifically, the resistance to ceftiofur, a critically important antimicrobial, was significantly higher at 46.9% in weaned piglets compared to other production stages. We also investigated the multi-drug resistance (MDR) ratio at each production stage. Most of isolates (92.2%) showed MDR. The difference in the MDR ratio for the 9 subclasses was particularly high in weaned piglets; suckling piglets (13.9%), weaned piglets (29.2%), grower pigs (17.6%), finisher pigs (14.2%) and sows (13.0%).

Conclusions: Through this study, it was found that weaned piglets in Korea exhibited higher AMR compared to other production stages. These findings could be useful in developing guidelines to reduce antimicrobial use and continuous monitoring of AMR on pig farms.

Disclosure: No significant relationships.

Keywords: antimicrobial resistance, multi-drug resistance, Escherichia coli, Pig, Production stage

SHIFT 02-014

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

INVESTIGATION OF ANTIMICROBIAL, ANTIBIOFILM ACTIVITY AND BIOACTIVE COMPOUNDS OF CHERRY STEM EXTRACTS

Lecture Title:

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Background and Aims: There are studies on the use of cherry (Prunus avium) stem extracts as an antimicrobial agent but none of them used Starks Gold cultivar. This study aims to obtain extracts from the stems of fruits collected in season from the Starks Gold cultivar and to examine the antimicrobial, antibiofilm, antioxidant effects, and bioactive compounds.

Methods: Cherry stem extracts were obtained using ethanol and methanol through microwave-assisted extraction (MAE). Extracts were stored at -20°C. Antimicrobial effect of the extracts was examined by agar well diffusion assay. Biofilm removal experiments were conducted to investigate the extracts' antibiofilm effect. Total phenolic content of the extracts was determined by Folin-Ciocalteu method, Total flavonoid content was determined by Aluminum Chloride method. Antioxidant activity was examined by their DPPH radical scavenging activities.

Results: Cherry stem extracts had no antimicrobial effect against Gram-negative bacteria. Meanwhile, both extracts showed antimicrobial activity against Gram-positive bacteria. It was observed that both extracts contain approximately 7 mg GAE/g of phenolic content and 0.4 mg QE/g flavonoid content. Both extracts showed antioxidant activity. It was observed that methanol and ethanol extracts removed 44% and 57% of the 48-hour-old biofilm formed by S. aureus, respectively.

Conclusions: The extracts obtained from cherry stems exhibit antimicrobial activity, particularly against Gram-positive bacteria, show biofilm removal effects, and possess antioxidant activity.

Disclosure: No significant relationships.

Keywords: Antibiofilm, antimicrobial, Cherry stem extract, Prunus avium

SHIFT 02-015

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

IDENTIFICATION OF HIGH-RISK LINEAGES OF APEC ISOLATED FROM POULTRY AND LAYER HENS IN BRAZIL: ANALYSIS OF ANTIMICROBIAL RESISTANCE

Lecture Title:

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Background and Aims: Avian pathogenic *Escherichia coli* (APEC) is a pathotype responsible for avian colibacillosis, and is associated with economic losses in chickens and poultry farming, increased use of antimicrobials and representing a potential zoonotic risk by foodborne transmission. The aim of this study was to investigate the presence of high-risk pandemic strains of APEC in Brazilian chicken and poultry farms.

Methods: 250 *E. coli* isolated from birds with colibacillosis in Brazil were subjected to PCR to assess the virulence genes *iroN, ompT, hlyF, iss* and i*utA*. The virulent strains were subjected to one-day-old chicks pathogenicity tests. After this screening, the high pathogenic index strains were subjected to whole genome sequencing on the Illumina Miseq platform. De novo assembly was carried out using the Unicycler v0.5 tool. Quality was assessed using Quast v.5.2. Bioinformatics analysis was carried out using the Centre for Genomic Epidemiology, Enterobase, Galaxy Europe and Pathogenwatch platforms.

Results: We identified ST117 (Phylogroup G- O78:H4) and ST349 (D- O86:H2) as the most pathogenic lineages of APEC in broilers farms. The O78 strain were resistant to the florfenicol, beta-lactams ($bla_{\text{CTX-M-55}}$) and quinolones, while the O86 strain were resistant to florfenicol, beta-lactams ($bla_{\text{TEM-1}}$), gentamicin and tetracycline. In the layer hens, the most pathogenic was the quinolone-resistant strain from ST95 (B2 O1:H7).

Conclusions: High-risk APEC strains are a current challenge for the poultry industry. These virulent strains show antimicrobial resistance to high-priority classes of drugs. Monitoring studies and the use of autogenous vaccines are necessary to reduce animal and human health risks.
Funding: FAPESP (2022/11917-1); CNPq (306396/2020-3).

Disclosure: No significant relationships.

Keywords: virulence, antimicrobial resistance, whole genome sequencing, Avian Pathogenic E. coli, APEC

SHIFT 02-016

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

INTESTINAL COLONIZATION WITH MULTIDRUG-RESISTANT ENTEROBACTERIACEAE AMONG AMBULATORY PATIENTS IN ABIDJAN

Lecture Title:

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Background and Aims: Multi-resistant Enterobacteriaceae (MRE) are increasingly around the world, and become a global health threat. In Africa, data on colonization on MRE are poor. This study aimed to determine the prevalence of colonization by MRE in outpatients, including their phenotypic characteristics.

Methods: A cross-sectional study was conducted from July to December 2023 at University Hospital of Treichville. Rectal swabs were collected from patients attending the general medical consultation ward selected by systematic random sampling. Enterobacteriaceae strains were identified by conventional methods and antibiotic susceptibility testing was carried out in accordance with the CA-SFM EUCAST 2019 guidelines. Data were analysed using Rstudio software version 4.2.1.

Results: Prevalence of MRE in rectal carriage was 32.72% (36/110). Extended Spectrum Beta Lactamase (ESBL) - producing Enterobacteriaceae represented 56.66% (34/60) and carbapenem resistant enterobacteria 3.33% (2/60). Among MRE carriers, the predominant age group was between 40 and older years; male represented 52.77% (19/36). *Escherichia coli* and *Klebsiella pneumoniae* were the common species at 61.66% (37/60) and 28.33% (17/60) respectively. Resistance associated to gentamycin (36.66%,22/60), ciprofloxacin (71.66%, 43/60) and cotrimoxazole (85.0%, 51/60) were observed. Risk factors like proximity to farms or bodies of water, pet ownership, recent hospitalization (less than 6 month), antibiotic use (less than 3 month), and chronic medical diseases were significantly associated with MRE rectal carriage (p= 0.03).

Conclusions: High rate of MRE in rectal carriage was observed. The knowledge of factors associated with MRE carriage helps to identify patients at risk to combat the spread of resistant bacteria within the healthcare system.

Disclosure: No significant relationships.

Keywords: Colonization, Abidjan, Multidrug Resistant Enterobacteriaceae, ESBL

SHIFT 02-017

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

COMPARATIVE ANALYSIS OF ANTIMICROBIAL RESISTANCE PROFILE OF ESCHERICHIA COLI ISOLATED FROM URINE SAMPLES FROM OUTPATIENTS AND INPATIENTS FROM SKOPJE, NORTH MACEDONIA

Lecture Title:

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Background and Aims: Urinary tract infections due to *Escherichia coli* are one of the leading causes for antibiotic prescription in both clinical and primary care. The appropriate selection of antimicrobial agent has a pivotal role in combating the antimicrobial resistance (AMR) worldwide. Our study aims to evaluate the difference of the antimicrobial resistance profile of urine isolates of *Escherichia coli* between outpatients and inpatients.

Methods: During September 2023-April 2024, 391 *Escherichia coli* isolates from positive urine culture from mid-stream urine samples from outpatients (253) and inpatients (138) routinely sent to our clinical microbiology laboratory were included in study analysis. AMR to clinically relevant antibiotics was evaluated according to the criteria of the European Committee on Antimicrobial Susceptibility Testing.

Results: Isolates from outpatients had significantly higher susceptibility rate in comparison to isolates from inpatients (p=0.003). Highest AMR rates between the two groups (outpatients vs. inpatients) were detected for ampicillin (25% vs. 72%), amoxicillin-clavulonic acid (10% vs. 34%), fluoroquinolones (16% vs. 43%) and trimethoprim/sulfamethoxazole (10% vs. 46%), while highest susceptibility rates were detected for imipenem/meropenem (100% vs. 100%), both amikacin and nitrofurantoin (99% vs. 93%), and gentamicin (92% vs. 77%). Significantly higher proportion of multi-drug resistant *Escherichia coli* isolates was identified among clinical samples (56%) compared to outpatients (25%); p<0.00001. Although all isolates from outpatients were susceptible, 2% of isolates from inpatients were resistant to ertapenem.

Conclusions: Considering the significant difference of *Escherichia coli* resistance rates between outpatients and inpatients improved implementation of AMR surveillance programs and enhanced antibiotic stewardship are warranted especially in the clinical setting.

Disclosure: No significant relationships.

Keywords: antimicrobial resistance, Escherichia coli, inpatients, outpatients

SHIFT 02-018

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

PHENOTYPIC AND GENOTYPIC CHARACTERISATION OF CARBAPENEMASE-PRODUCING KLEBSIELLA PNEUMONIAE ISOLATED FROM TERTIARY HOSPITAL ENVIRONMENT IN ABIDJAN, IVORY COAST

Lecture Title:

Meilleure Nathalie Kouadio[1], Ivanne Alexia Dechy Yapi[1,2], Sodji Emilie Karen N'Goran[1], Gninissemet Armel Joel Bahan[1,2], Maky Diallo[3], Yves Gontran Lobah[4], Mohamed Tohé[5], Marina Coulibaly-Diallo[1], Adele Kacou N'Douba[1,2]
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Background and Aims: Klebsiella pneumoniae carbapenemase (KPC) producing is an emerging pathogen in healthcare-associated infections (HAIs). The aim of this study was to assess the prevalence, phenotypic profiles and related genes of carbapenem-resistant Klebsiella pneumoniae (CRKP) among hospital environment samples.

Methods: The study was cross-sectional, processed at September 2023 in the medical department at Angre's University Hospital. A total of 174 swabs were collected from inanimate surfaces in the hospital environment. All samples were cultured and K. pneumoniae strains were identified by conventional microbiological methods. Antibiotic susceptibility was tested using the disk diffusion Kirby-Bauer method. Carbapenem resistance was determined based on imipenem, meropenem, and ertapenem zones of inhibition, as well as minimum inhibitory concentrations (MICs). Resistance genes were assessed by multiplex PCR targeting the blaKPC, blaIMP, blaNDM, blaVIM, and blaOXA-48 genes.

Results: Among 108 bacterial strains, Klebsiella pneumoniae represented 24.07 % (26/108), and 23.07% (6/26) of them were carbapenem-resistant. Rate was higher in the ICU (13/26; 50%). KPC isolates were also resistant to tetracycline (100%), gentamycin (83.33%), cotrimoxazole (83.33%) and ciprofloxacin (44.44%). All strains of CRKP (100%) were phenotypically metallo-β-lactamase (MBL) producers. By genotypic tests, the gene blaIMP was only expressed, with 100%. However, blaKPC, blaVIM, blaNDM, and blaOXA-48 were not detected.

Conclusions: This study showed that the environment of our hospitals is contaminated with KPC and it emphasizes the importance of prevention and infection control strategies.

Disclosure: No significant relationships.

SHIFT 02-019

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

HYPOCHLOROUS ACID AS AN EFFECTIVE FORCE AGAINST THE BACTERIAL BIOFILM IN COSMETIC INDUSTRY.

Lecture Title:

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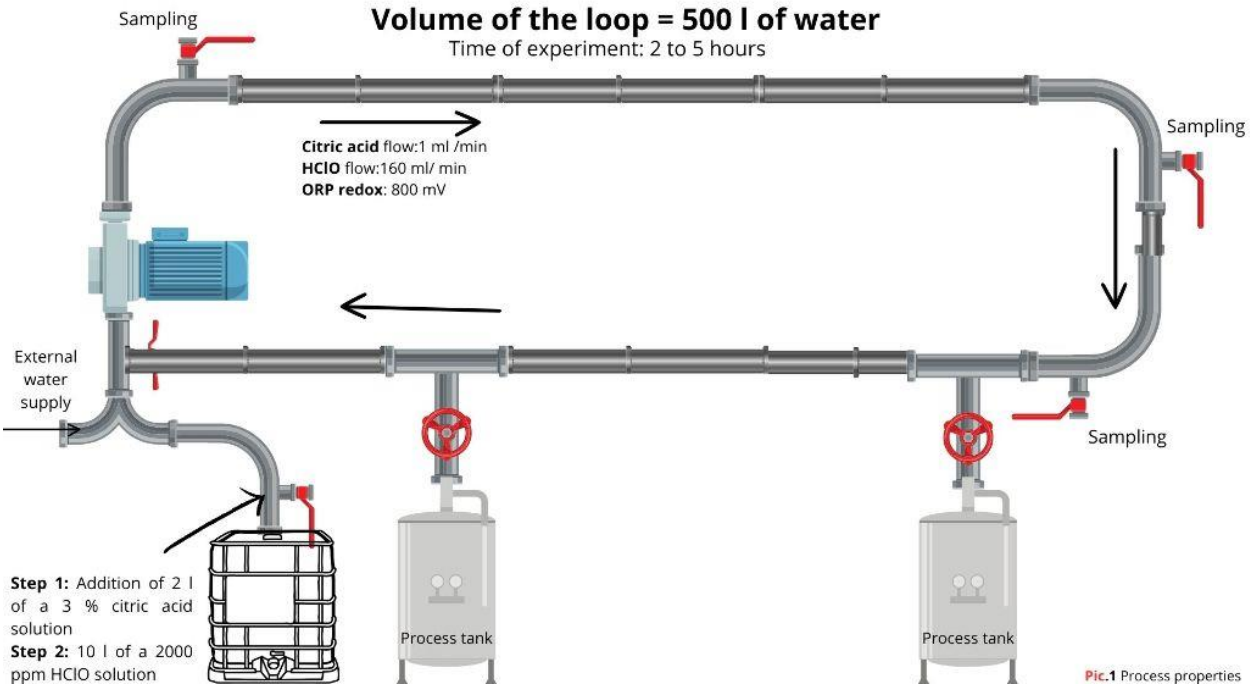
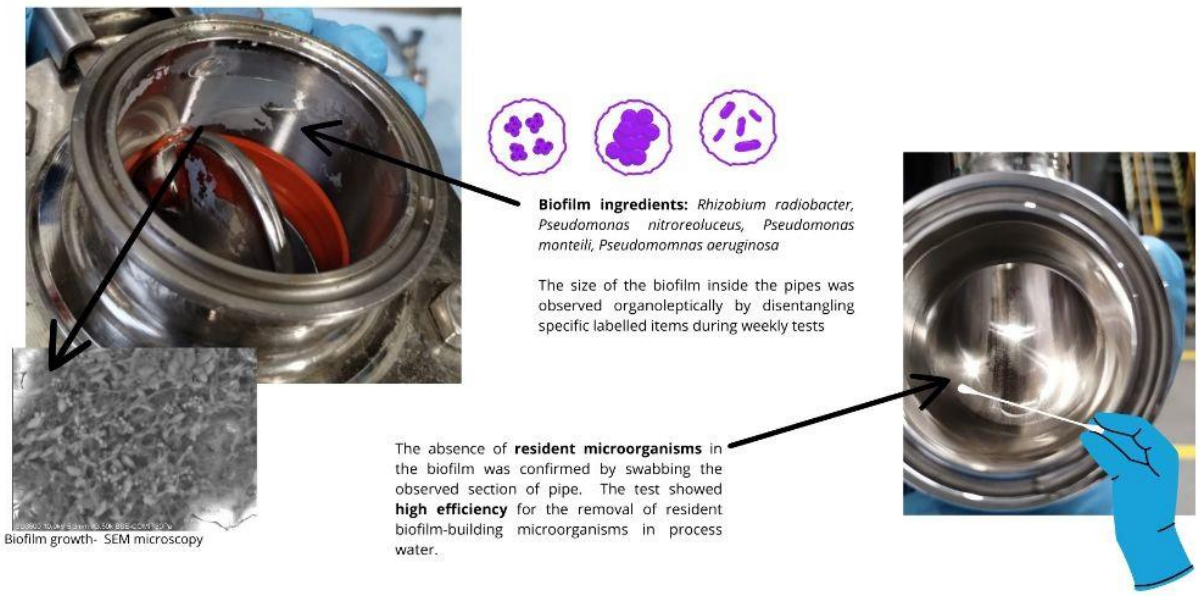
Background and Aims: Bacterial biofilm has been an indispensable problem in manufacturing plants, especially in process water pumping systems. The production plant, on which this study is based is a cosmetic company. The aim of the study was to demonstrate the effectiveness of shock disinfection of a water pumping system using hypochloric acid solution (HClO) and its biofilm removing efficiancy.

Methods: The HClO solution was obtained by salt electrolysis -2000 ppm. Testing was carried out on a technical scale after checking the effectiveness of HClO on a bacterial biofilm grown in the laboratory. The laboratory test material was made of 316Ti steel - construction material of water pumping system. The biofilm was grown by using the mixture of bacteria isolated from the production environment. The process was run for six months, each week on Monday. Pic.1 shows steps and process parameters. Evaluation of disinfection and biofilm reduction effectiveness was involved by: checking the level of contamination of the water and taking swabs from disconnected plant components and SEM
microscopy.



Pic.1 Process properties

Results: The level of contamination of the water, decreased from an uncountable level in 1 ml through $3*10^3$CFU/ml, finally reaching 0 CFU/ml. The disinfecting effect initially lasted for two days, finally extending to a week. Picture 2 shows the biofilm removal efficiency.



Pic.2 Biofilm removal efficiancy

Conclusions: The test showed high efficiency for the removal of resident biofilm-building microorganisms in process water. In addition, the solution is environmentally friendly, allowing the solution to be discharged into the combined sewer system, as there are no hazards associated with the use of HClO.

Disclosure: No significant relationships.

Keywords: hypochlorous acid, water treatment, cosmetic industry, disinfection

SHIFT 02-020

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

MOLYBDENUM CLUSTERS AS A NOVEL ACTIVE INGREDIENT FOR TOPICAL PHOTODYNAMIC THERAPY

Lecture Title:

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Background and Aims: New antibiotic approaches are urgently needed to combat the rising threat of antimicrobial resistance. Photodynamic therapy (PDT) emerges as an innovative stratagem centered on the photoinactivation of pathogenic bacteria through the utilization of materials termed photosensitizers. These photosensitizers engender a profusion of reactive oxidative species upon exposure to visible light. Owing to the substantial induction of oxidative stress affecting various components within prokaryotic cells, PDT has demonstrated a diminished proclivity to incite resistance within microbial populations.

Methods: Herein, we present antibacterial photosensitizers predicated on octahedral molybdenum cluster complexes (Mo_6) activated by blue light. Mo6 were formulated into semi-solid topical pharmaceutics containing three distinct types of Mo_6clusters - ($Na_2[Mo_6I_8(OPOPh_2)_6]$), $[Mo_6I_8(OCOC_4H_8PPh_3)_6]Br_4$, and $Na_2[Mo_6I_8(cholate)_6]$) as potential novel actives for cosmetics purposes.

Results: Cream formulations were developed utilizing natural deep eutectic solvents as carriers of these active compounds and tested for their stability, characterization, viscosity, skin penetration, and singlet oxygen formation. After that, non-toxicity and safety were confirmed using *in vitro* testing on HaCaT cell lines. Finally, antimicrobial PDT was conducted to demonstrate efficacy against *Cutibacterium acnes* and *Staphylococcus aureus* as the most prevalent skin bacterial pathogens utilizing blue light and daylight as a source of exposure.

Conclusions: This application offers a novel approach to combat skin infections without resorting to antibiotics, thereby mitigating the contribution to antibiotic resistance. Additionally, this work may pave the way for advancements in the field of photodynamic therapy in dermatology and cosmetic science.

Disclosure: No significant relationships.

Keywords: photodynamic therapy, cosmetics, new antibacterials, oxidative stress, dermatology

SHIFT 02-021

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

ANTIBIOTIC RESISTANCE IN ACINETOBACTER SPP. FROM CZECH CATTLE FARMS – A MULTIPHASIC APPROACH

Lecture Title:

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Background and Aims: Effective combating antimicrobial resistance requires the integration of medical, environmental and agricultural aspects. Notably, feces/manure from antibiotic-treated livestock are important sources of antibiotic resistant bacteria. This study aims to assess the risk posed by *Acinetobacter* spp. present in cattle feces in terms of antibiotic resistance dissemination.

Methods: Our work integrates a cultivation approach, genomics and metagenomics, all conducted on composite fecal samples from 28 Czech cattle farms with contrasting levels of antibiotic use. Taxonomically identified *Acinetobacter* strains (n=284) were tested for sensitivity to 19 antibiotics. Genomes of chosen strains and metagenome-assembled genomes (MAGs) from *Acinetobacter* enrichment cultures were scrutinized for the presence of acquired antibiotic resistance genes (ARGs) and their genetic surroundings.

Results: *A. indicus* and *A. pseudolwoffii* were the most commonly identified *Acinetobacter* spp. in cattle feces based on both isolated strains and metagenomics. Strain-specific resistance to streptomycin, sulfonamides, trimethoprim and tetracycline were most common, but not directly related to on-farm antibiotic use. *A. baumannii* was scarce in cattle feces, with two strains resistant to kanamycin. Genomic analyses mostly focused on putative novel species, representing ≈40% *Acinetobacter* strains. They revealed the presence of *aad27*, *strA*, *strB, sul2* and *tet*(Y) genes, in various combinations of insertion sequences, transposons and plasmids. *Acinetobacter* MAGs in addition harbored *tet*(39), *clmB1* and OXA-58.

Conclusions: *Acinetobacter* spp. from cattle feces represent a source of mobilizable ARGs. The mobile genetic element arsenal of acinetobacters and presence of clinically relevant ARGs (e.g. carbapenemase OXA-58) call for prudent antibiotic use in farms and proper manure management practices.

Disclosure: No significant relationships.

Keywords: Acinetobacter, cattle feces, Genomics, Metagenomics, Antibiotic resistance

SHIFT 02-022

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

EXPLORING BACILLUS STRAINS FOR POTENTIAL BIOCONTROL OF CITRUS SCAB AND GREEN MOLD

Lecture Title:

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Background and Aims: *Elsinoe fawcettii* and *Penicillium digitatum* are significant pathogens affecting citrus. *E. fawcettii*, which causes scab disease, produces elsinochrome, a phytotoxin. *P. digitatum*, responsible for citrus green mold, causes the greatest post-harvest loss of citrus. These diseases are mostly controlled by pesticides, which are toxic and harmful to the environment. This study explores the use of antagonistic bacteria as effective alternatives to synthetic fungicides for managing these citrus diseases.

Methods: Several bacterial isolates from soil and food were screened for their antifungal activity using the disk agar diffusion method. Their extracellular enzyme activities were detected by patching bacteria on LB agar plates supplemented with different substrates. Chitinolytic activity was estimated by the dinitrosalicylic acid (DNS) method using colloidal chitin as a substrate.

Results: Of the 60 bacterial strains, two showed antifungal activities against *E. fawcettii* and *P. digitatum*, marking them as potential control agents. These strains, *Bacillus subtilis* JBRI-B21-622 and *B. amyloliquefaciens* JBRI-B21-046, demonstrated the ability to produce several extracellular enzymes such as proteases, amylase, cellulases, and lipases. These two strains also produce chitinase, a hydrolytic enzyme that breaks down the glycosidic bonds in chitin, a major component of fungal cell walls.

Conclusions: Further research is necessary to fully understand the antifungal mechanisms of these strains. However, these results suggest that *B. subtilis* JBRI-B21-622 and *B. amyloliquefaciens* JBRI-B21-046 offer environmentally friendly solutions to control citrus diseases caused by *E. fawcettii* and *P. digitatum*, reducing dependency on chemical fungicides. This research was financially supported by Jeju Special Self-governing Province.

Disclosure: No significant relationships.

Keywords: antifungal activity, Biological control, Citrus Diseases, Bacillus Strains, Chitinase

SHIFT 02-023

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

PLANT VERSUS ANIMAL ISOLATE - SIMILARITIES AND DIFFERENCES IN ACTION AGAINST CANDIDA ALBICANS

Lecture Title:

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Background and Aims: Plant species of the genus Sida are widely distributed due to their medicinal properties. Which is why they are used as a sources of compounds with antibacterial, anti-inflammatory, and antioxidant properties. *Sida hermaphrodita* is a perennial herb with potential economic importance. However, there has been no information about its antimicrobial properties so far.

Methods: Treated and non-treated cells of clinical *Candida albicans* strain were analysed with microscopic, spectroscopic and flow cytometry methods (NCNgrants 2020/37/B/NZ7/00763; 2023/07/X/NZ3/00661).

Results: Microscopic analysis showed that the crude extract of *S. hermaphrodita* seeds caused cell deformation, disruption of cell division, and significant decrease in the metabolic activity of fungal cells leading to cell death via necrosis and autophagy pathway. The effect of the seed extract was compared with the protein-polysaccharide complex extracted from the coelomic fluid of the earthworm *Dendrobaena veneta*. Similar cell morphological changes were observed, consisting in the formation of multi-cell aggregates. Apoptosis and autophagy were the main pathways of cell death. Observations indicated that the target of action of both preparations is the cell wall of the analyzed fungi, which was confirmed by FTIR analyses. Analyses using flow cytometry confirmed the increased mortality of yeast cells after the action of the analyzed preparations.

Conclusions: The observations indicated the effective action of the analyzed extracts on *C. albicans* clinical strain, focusing on the destruction of the cell wall, which is a desirable feature of a potential antifungal drug. The lack of toxicity of both extracts to human skin fibroblast cells qualifies this compounds for further biomedical research.

Disclosure: No significant relationships.

Keywords: Candida albicans, earthworms, antifungal activity, plants

SHIFT 02-024

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

EFFECT OF 5-AMINOLEVULINIC ACID-MEDIATED PHOTODYNAMIC THERAPY ON FUSOBACTERIUM NUCLEATUM SUBSPECIES

Lecture Title:

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Background and Aims: Periodontal disease, a major global cause of tooth loss, results from the synergistic interactions between subgingival dental biofilm and periodontal tissues. *Fusobacterium nucleatum* plays a crucial role in the development of these biofilms and is divided into four subspecies—*animalis*, *nucleatum*, *polymorphum*, and *vincentii*—each known to vary in virulence and biofilm-forming capabilities. Photodynamic therapy (PDT) using 5-aminolevulinic acid (5-ALA) (ALA-PDT) represents a minimally invasive approach that employs 5-ALA, a precursor to porphyrin-type photosensitizers, and light of specific wavelengths to target microorganisms effectively without promoting antimicrobial resistance. Our previous research has demonstrated that ALA-PDT with 5-ALA phosphate can successfully disinfect *F. nucleatum*. This study investigates the differential impacts of ALA-PDT on the subspecies of *F. nucleatum*

Methods: We incubated *F. nucleatum* with 5-ALA phosphate for 20 hours followed by exposure to 635 nm LED light, measuring outcomes through colony forming unit (CFU) counts, and porphyrin extraction and quantification.

Results: The findings reveal that ALA-PDT markedly decreases CFU counts across all subspecies. However, there are notable differences in the bactericidal effects among the subspecies, which correlate with the levels of porphyrin production.

Conclusions: These findings suggest that while ALA-PDT can eradicate *F. nucleatum* broadly, enhancing porphyrin induction may improve its efficacy against *F. nucleatum*.

Disclosure: T.N. and O.T. signed a joint research agreement with SBI Pharmaceuticals Co., Ltd. relevant to this article.

Keywords: photodynamic therapy, 5-aminolevulinic acid, Fusobacterium nucleatum, Fusobacterium nucleatum subspecies

SHIFT 02-025

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

QUATERNARY AMMONIUM SALTS AS BIOCIDES - DIFFERENCES IN MICROBICIDAL EFFICACY ON RESISTANT STRAINS

Lecture Title:

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Background and Aims: Quaternary ammonium salts (QAS) are a structurally diverse group of substances that have long been used as highly effective antimicrobial agents in the form of disinfectants or antiseptic preparations. Despite the relatively non-specific mechanism of action (disruption of cell membranes) caused by the presence of quaternary nitrogen and at least one long lipophilic chain, the differences between individual QAS groups are quite significant.

Methods: The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were measured using the microdilution broth method for the basic evaluation of the antimicrobial effectivity of novel compounds. Furthermore, the Calgary biofilm assay was used to determine the minimum biofilm eradication concentration.

Results: In this study, we specifically compared the effect of several QAS from different groups on the bactericidal efficiency of the collection strain of Pseudomonas aeruginosa in comparison with several isolates of the same strain from the clinical operation of the hospital. Likewise, there are significant differences in the sensitivity of different isolates of the same strain, where collection strains show the highest sensitivity and clinical isolates resistant to antibiotics the lowest.

Conclusions: Over the past 10 years, our group has prepared more than 180 new substances of the QAS type from several of the most used groups in practice, divided by structure. All substances were screened against a wide range of microorganisms (bacteria, fungi, viruses) and the influence of the structure and effectiveness of individual groups as well as differences in sensitivity within one strain isolated from various samples of clinical use was monitored.

Disclosure: No significant relationships.

Keywords: Biocides, antimicrobial, Resistance, quaternary ammonium salts, synthesis

SHIFT 02-028

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

DECIPHERING THE GENETIC AND PHENOTYPIC LANDSCAPE OF MACROLIDE RESISTANCE IN ALIARCOBACTER BUTZLERI

Lecture Title:

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Background and Aims: Widely distributed, *Aliarcobacter butzleri* is recognized as a potential public health concern due to its transmission among humans and animals. While most infections are self-limiting, severe or persistent cases may require antibiotic therapy, with macrolides being commonly recommended. However, the prevalence of multidrug-resistant strains underscores the complexity of managing *A. butzleri* infections, particularly given the variable macrolide resistance rates reported and the unknown nature of its mechanisms.

Methods: Aiming to gain insights on this topic and unravel the phenotypic and genotypic changes associated with resistance, a fluctuation assay was used to determine the mutation rate in *A. butzleri* strain DQ40A1, isolated from food products, followed by comprehensive analyses of derived clones: profiling cross-resistance and collateral sensitivity, elucidating genetic causes of resistance via Sanger sequencing of the transcription repressor of the AreABC efflux pump (*areR*), and functionally correlating findings through ethidium bromide accumulation assays. Additionally, the impact of erythromycin resistance on bacterial fitness and virulence was assessed, encompassing growth kinetics, motility, and biofilm formation.

Results: The native strain was classified as weakly hypermutable, and the results in the resistant strains point to mutations in the repressor *areR* causing an overexpression of the AreABC efflux pump. This resulted in decreased ethidium bromide accumulation, supporting the erythromycin reduced susceptible phenotype and cross-resistance profiles. Importantly, some resistant strains incurred a fitness cost, manifested as altered growth dynamics and compromised virulence traits.

Conclusions: Overall, this study elucidates macrolide resistance mechanisms in *A. butzleri* and underscores the intricate interplay between antibiotic resistance, bacterial fitness, and pathogenicity in this species.

Disclosure: No significant relationships.

Keywords: Aliarcobacter butzleri, macrolide resistance mechanisms, efflux pumps, virulence, fitness

SHIFT 02-029

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

INSIGHTS INTO ANTIMICROBIAL RESISTANCE EVOLUTION IN ALIARCOBACTER BUTZLERI UNDER SUBCLINICAL CIPROFLOXACIN EXPOSURE

Lecture Title:

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Background and Aims: Reports of multidrug-resistance rates in *Aliarcobacter butzleri*, an emerging enteropathogen, raise concerns about compromised treatment options for infections caused by this microorganism. Fluoroquinolones are one of the recommended classes of antibiotics for infections by this bacterium. However, ciprofloxacin is classified by the World Health Organization as a critically important antimicrobial, designated within the Watch category. Although antimicrobial resistance has traditionally been associated with high therapeutic concentrations of antibiotics, recent evidence suggests that subclinical concentrations can drive the selection of multidrug-resistant mutants while also influencing bacterial physiology and virulence.

Methods: Aiming to unveil the evolutionary pathways of *A. butzleri* in presence of subclinical ciprofloxacin concentrations and associated trade-offs, three strains from human, food and environmental origin were phenotypically characterized regarding resistance to ciprofloxacin and submitted to a 12-day ciprofloxacin-adaptative laboratory evolution in its presence. Following experimental evolution, potential genotypic and phenotypic implications within a One Health framework were explored. Populations' susceptibility to ciprofloxacin and cross-resistance profiles to antibiotics, biocides, heavy metals, and ethidium bromide were evaluated, as well as the underlying resistance mechanisms. Furthermore, mutants isolated from evolved populations were examined for potential altered fitness costs and virulence factors.

Results: Notably, multidrug-resistant mutants emerged even at concentrations below those found in serum of individuals not undergoing antibiotic therapy or in ciprofloxacin-polluted environments, with changes in the susceptibility to ethidium bromide, suggesting the involvement of efflux pumps activity.

Conclusions: From a One-Health perspective, these findings underscore the relevance of subclinical antibiotic concentrations in worsening the antibiotic resistance crisis, particularly concerning environmental pathogens like *A. butzleri*.

Disclosure: No significant relationships.

Keywords: Aliarcobacter butzleri, Antibiotic resistance, One Health, experimental evolution, subclinical ciprofloxacin concentrations

SHIFT 02-030

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

RAPID DETECTION OF RESISTANT BACTERIA BY MEASURING BACTERIA GROWTH FOR GRAM-POSITIVE COCCI USING SCANNING ELECTRON MICROSCOPY

Lecture Title:

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Background and Aims: Bacterial drug resistance remains to be a worldwide problem. There is a need to rapidly examine the antimicrobial efficacy against organisms causing infections. We developed a rapid evaluation method of the effect of antimicrobial agents by quantifying bacterial growth after exposure to antimicrobial agents using tabletop scanning electron microscope (SEM).

Methods: Sensitive and resistant strains of *Staphylococcus aureus* were used as the model material. A 10^5 CFU/mL bacterial suspension was exposed to Vancomycin at EUCAST MIC breakpoint. Samples were incubated for 60 minutes and prepared for SEM observation in 15 minutes. Bacteria untreated with antimicrobials were used as a control. The bacterial images were captured using a tabletop SEM. Analysis was performed by collecting to the optimal area size for bacterial concentration and differentiating growth by binarization of the SEM images.

Results: Following exposure to Vancomycin, the bacteria area of susceptible strain was low compared to control bacteria at 60 minutes. On the other hand, the bacteria area of resistant strain was comparable to that of controls. Thus, bacterial growth at low concentrations in the early stages of growth can also be rapidly evaluated.

Conclusions: Quantification of bacteria area of 60 minutes after incubation with Vancomycin using SEM showed significant differences in the bacteria area of susceptible and resistant bacteria. This method could be used to assess bacterial growth. This method has the potential to detect resistant bacteria in a shorter incubation time compared to conventional evaluation methods such as absorbance detection.

Disclosure: This study was funded by Hitachi High-Tech Corporation

Keywords: staphylococcus aureus, Vancomycin, Scanning electron microscopy (SEM), Antimicrobial Susceptibility, Image Analysis

SHIFT 02-033

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

THE METHYLGLYOXAL AS ACTIVE SUBSTANCE IN WOUND DRESSING MATERIALS BASED ON FISH SKIN COLLAGEN.

Lecture Title:

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Background and Aims: Damaged skin loses its natural protective mechanism, creating an active portal for pathogens and microorganisms. Wound dressing materials based on collagen absorb large amounts of body fluids and keep moist microclimate of a wound and stimulate a faster wound healing. The aim of this research was to obtain and characterized the biological properties of collagen material modified with beta-glucan, along with the addition of active compounds from Manuka honey as antimicrobial agents.

Methods: Collagen was obtained from fish skin (*Hypophthalmichthys molitrix)*. MIC and MBC values of methylglyoxal (MGO) were determined. The materials were prepared using the casting method, with 0.125 and 0.25 mg/cm^2 MGO concentrations. Antimicrobial properties were obtained by following ISO 22196 standard. Biocompatibility studies were performed on HaCaT cultures.

Results: MIC and MBC values for MGO against *P. aeruginosa* and *E. coli* were 19.53 µg/ml, while against *S. aureus*, MIC was 9.77 µg/ml and MBC was 19.53 µg/ml. According to the ISO 22196 procedure, only materials with MGO concentration of 0.25 mg/cm^2 exhibited a reduction index above 2 ($R \geq 2$), qualifying material as bactericidal. All prepared materials were non-toxic to HaCaT cells, thus classifying the materials as biocompatible with skin cells.

Conclusions: The material with 0.25 mg/cm^2 of MGO was characterised as antimicrobial against pathogens and biocompatible simultaneously. Consequently, these findings could serve as the foundation for further advanced research on the utilization of collagen/beta-glucan and methylglyoxal materials as dressing materials. **The research was financed by the National Science Centre, Poland, Grant nr 2018/31/N/ST8/01509.**

Disclosure: No significant relationships.

Keywords: antimicrobial, wound dressing, methylglyoxal, collagen

SHIFT 02-034

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

SINGLE MOLECULE IMAGING OF NECTRIATIDE, A POTENTIATOR OF AMPHOTERICIN B, USING RAMAN MICROSPECTROSCOPY

Lecture Title:

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Background and Aims: We discovered that the nectriatide (**Nec**) has function as a potentiator of the antibiotics amphotericin B (AmB), where it enhances the antifungal activity of AmB. Furthermore, a linear intermediate (**linear-Nec**) significantly enhances the antifungal activity of AmB. Experiments using a chemical probe led to the hypothesis that Nec exhibits antimicrobial activity by binding to ergosterol present in the cell membrane. In this study, we elucidate the detailed action mechanism of Nec by using Raman microspectroscopy to observe the localization of unlabeled ergosterol, linear-Nec, and AmB in living yeast cells.

Methods: We treated *Saccharomyces cerevisiae* with **linear-Nec** (64 μg/mL) and AmB (0.25 μg/mL) at 30°C for one hour. Using Raman microspectroscopy, we detected ergosterol, **linear-Nec** and AmB within yeast cells. Multivariate curve resolution-alternating least squares (MCR-ALS) analysis was applied to visualize these molecules.

Results: In *S. cerevisiae*, **linear-Nec** and AmB were found to be abundantly localized in areas where ergosterol is abundant, revealing co-localization of these compounds. AmB is known to assemble into seven-molecule ion channels with ergosterol. Therefore, it is proposed that Nec enhances AmB's antifungal activity by stabilizing or promoting the formation of these ion channels.

Conclusions: Raman microspectroscopy visualized the co-localization of ergosterol, linear-Nec, and AmB in *S. cerevisiae* without labeling. Our findings demonstrate that this co-localization significantly enhances the antifungal activity of AmB.

Disclosure: No significant relationships.

Keyword: fungi, antifungal medicine, peptide, amphotericin B, a potentiator of amphotericin B

SHIFT 02-035

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

ENZYME PROFILE AND THE ANTIBIOTIC RESISTANCE PROPERTIES OF LACTOBACILLI DERIVED FROM BREAST MILK.

Lecture Title:

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Background and Aims: The gut microbiota is crucial for the overall health of the host, and lactic acid bacteria (LAB) have become widely recognized for their role in promoting health. This study assessed the antibiotic susceptibility of LAB isolated from human breast milk samples. The aim of this study was also to isolate, identify, and characterize certain lactic acid bacterial strains from human milk as potential probiotics with antimicrobial activity against some human pathogenic strains. Additionally, we determined some important enzymatic activities among the isolated strains.

Methods: Antibiogram tests were conducted to determine the sensitivity or resistance of LAB to conventional antibiotics. The standard disk diffusion analysis was employed to assess the antibiotic sensitivity pattern. Candida albicans (ATCC 10231), Staphylococcus aureus (ATCC 12228), and E. coli (ATCC 25922) were used to determine the antimicrobial activity. We used the Bolog system to determine different lactobacillus strains' ability to utilize many different substrates. In this way, we can predict the different enzyme activities and metabolisms of the studied strains.

Results: We isolated and identified 12 strains of lactobacilli, with a large portion of them proving to be sensitive to the antibiotics under study. One of the strains exhibited antibacterial activity and also demonstrated variations in the assimilation of different substrates.

Conclusions: Further in-depth studies on the antibiotic resistance of probiotic strains are necessary to ensure their safe use. Acknowledgment: This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project BG-RRP-2.004-0001-C01.

Disclosure: No significant relationships.

Keywords: Lactobacillus, probiotic, antibiotic resistance, Breast milk

SHIFT 02-036

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

UNRAVELLING ANTIMICROBIAL RESISTANCE: ENTEROCOCCI'S CIRCULATION ACROSS ONE HEALTH SECTORS

Lecture Title:

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Background and Aims: *Enterococcus* are frequent causes of healthcare-infections, being vancomycin-resistant *E. faecium* considered a serious health priority due to limited therapeutic options. This study aimed to recover enterococci from various One Health (OH) sectors and evaluate antimicrobial resistance (AMR).

Methods: In total, 781 samples were collected (2022-2024) from healthy/ill humans and animals, healthcare-settings, and environment. Following processing using selective media with/without vancomycin, and RAPD-comparison, distinct isolates were identified with molecular methodologies. A collection of 439 enterococci was subjected to AMR testing (CLSI guidelines) against 12 clinically relevant antibiotics.

Results: The human sector revealed high levels of quinupristin-dalfopristin resistance (66%; *E. faecalis*: 92%), rifampicin (38%), erythromycin (46%), and vancomycin (23%), with moderate resistance to streptomycin and levofloxacin (14%). Vancomycin resistance was particularly high in isolates from healthcare surfaces (40%) and healthy humans (31%), surpassing that of clinical isolates (17%). The animal sector showed higher rifampicin resistance (56%), while resistance to other antibiotics, including vancomycin, was lower (8%; no resistance in healthy pets). In the environment, elevated levels of vancomycin (33%) and teicoplanin (21%) resistance were observed, particularly among isolates from food (29% and 17%, respectively) and food-related surfaces (60% and 48%). Resistance to other clinically relevant antimicrobials, including β-lactams, oxazolidinones, tetracyclines, and aminoglycosides, was low across sectors. Significant differences in AMR profiles were observed between sectors.

Conclusions: This study underscores the importance of surveillance for antimicrobial resistance within and across OH sectors. Acknowledgements: FCT IP Portugal, through projects UIDB/00276/2020 (CIISA), LA/P/0059/2020-AL4ANIMALS, UIDB/04462/2020 and UIDP/04462/2020 (iNOVA4Health), and LA/P/0087/2020 (LS4FUTURE). JMarques holds a doctoral fellowship (BIPM CIISA 2/2022).

Disclosure: No significant relationships.

Keywords: Enterococcus, One Health, antimicrobial resistance, Surveillance

SHIFT 02-037

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

CHARACTERIZATION OF A MULTIDRUG RESISTANT CITROBACTER PORTUCALENSIS CO-PRODUCING NDM-1, KPC-2 AND OXA-48 CARBAPENEMASES FROM URBAN WASTEWATERS

Lecture Title:

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Background and Aims: Wastewaters could be used to track the spread of several pathogens, including multidrug resistant (MDR) bacteria and antimicrobial resistance mechanisms. Carbapenem-resistant *Enterobacterales* (CRE) represents one of the most difficult-to-treat MDR pathogens emerging also as a major clinically relevant issue. The aim of this work was the phenotypical and genomic characterization of a MDR *Citrobacter portucalensis* co-producing class A, B and D carbapenemases.

Methods: 50 mL collected from an urban wastewater treatment plant (Florence, Italy), were centrifuged at 4000 rpm for 10 minutes, then 10 µL of pellet were cultured on CRE-selective agar medium (ChromID® CarbaSmart, bioMèrieux). Bacterial identification was performed using MALDI-ToF mass spectrometry. Presence of acquired β-lactamases genes was assessed with multiplex real-time PCR and confirmed with short-reads WGS (Illumina). Antimicrobial susceptibility testing was performed using broth microdilution (BMD) and interpreted according to EUCAST clinical breakpoints (v14.0, 2024).

Results: An ST526 *C. portucalensis* isolate was retrieved from a wastewater sample collected in December 2023. Presence of $bla_{\text{NDM-1}}$, $bla_{\text{KPC-2}}$, and $bla_{\text{OXA-48}}$ genes was assessed with real-time PCR and WGS. BMD showed a highly resistant phenotype, including resistance to cefiderocol (MIC >64 mg/L) and novel β-lactams/β-lactamases inhibitors combinations (meropenem/vaborbactam, 32 mg/L; imipenem/relebactam >8 mg/L), retaining susceptibility only to amikacin and colistin (MIC ≤4 and ≤0.5 mg/L, respectively).

Conclusions: The presence of an MDR isolate co-producing several carbapenemase genes in urban wastewaters is of concern. Environmental spread of MDR bacteria might be an important reservoir of clinically relevant resistance mechanisms, denoting the importance of wastewaters surveillance studies.

Disclosure: No significant relationships.

SHIFT 02-039

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

GENOMIC ANALYSIS OF DIARRHEA-CAUSING BACTERIA IN KOLKATA, INDIA IN 2019.

Lecture Title:

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Background and Aims: India is considered a major source of traveler's diarrhea, and drug-resistant strains are widespread. However, genomic analysis of diarrheal disease-causing bacteria is limited, and the prevalence of genes involved in resistance and virulence, and the relationship to the global epidemic strain, remain unknown. In this study, whole genome analysis of diarrhea-causing bacteria isolated from diarrhea patients in Kolkata, India, was conducted to determine their genetic characteristics.

Methods: In 2019, ETEC (n=40), *Shigella* (n=111), and *Campylobacter* (n=101) isolated from diarrhea patients at two hospitals in Kolkata were whole genome sequenced for phylogenetic analysis, resistance and virulence factor analyses.

Results: The strains showed diverse lineages (*Campylobacter*, 29 ST types; ETEC, 20 ST types), except for *Shigella* (10 ST types), known to have low genetic diversity. Although some of the strains were consistent with the globally endemic strains, most of them were classified into different lineages. QRDR mutations conferring fluoroquinolone resistance were frequently observed in all bacteria (*Campylobacter*, 83%; ETEC, 73%; *Shigella*, 91%). In *Campylobacter*, 23S rRNA mutations conferring macrolide resistance, an important therapeutic agent, were also detected in 15% of isolates. In ETEC and *Shigella*, multidrug-resistant strains carrying resistance genes to more than three classes of antibiotics were identified (*Shigella* 64%, ETEC 30%).

Conclusions: Although the global spread of strains from India is less probable, the prevalence of a highly resistant and genetically diverse diarrhea-causing organism in a region with a high incidence of traveler's diarrhea may transmit a more challenging organism to treat, suggesting the importance of continued detailed surveillance using whole-genome analysis.

Disclosure: No significant relationships.

Keywords: Shigella, ETEC, Campylobacter, Next generation sequencing, India

SHIFT 02-040

Topic: ***AS02. Antimicrobials and antimicrobial resistance***

IMPORTED FISHERY PRODUCTS CONTRIBUTE TO THE SPREAD OF CARBAPENEM-RESISTANT BACTERIA ACROSS COUNTRIES.

Lecture Title:

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Background and Aims: There is an increasing concern regarding the emergence and spread of antibiotic-resistant bacteria (ARB) that severely impact the global public health system. Among the resistant bacteria, carbapenem-resistant Enterobacteriaceae, are designated priority 1 by the WHO and are considered one of the most important resistant bacteria to pay attention to. The contamination of foodstuffs with ARB should also be noted, as they can directly lead to human carriage through consumption. This study aims to determine the prevalence of carbapenem-resistant Enterobacterales in fishery products.

Methods: We purchased 103 fishery products in Vietnam and 15 Southeast Asian fishery products imported to Japan. The gut contents were aseptically removed, mixed with buffered peptone water, and incubated. The bacterial broth was spread onto CHROMagar ECC containing meropenem. After Enterobacterales were selected, antibiotic susceptibility testing was performed. Bacterial identification and genotyping were performed using 16S rRNA and multiplex PCR. Moreover, whole genome sequencing and conjugation assays were performed.

Results: Culture results showed that bacteria were detected in 11.7% of Vietnamese and 6.7% of imported fishery products. A total of 146 and 31 strains were isolated, and antibiotic susceptibility testing revealed 22 carbapenem-resistant strains in Vietnamese and one in imported fishery products. These resistant bacteria were *Escherichia coli*, *Enterobacter*, *Klebsiella*, and *Citrobacter*. These carbapenem-resistant Enterobacterales harboured NDM-1, -4, -5, OXA-48, and KPC.

Conclusions: Plasmid-mediated carbapenem-resistant Enterobacterales harbouring NDM, OXA, and KPC were isolated from Vietnamese fisheries products, and carbapenem-resistant *Enterobacter cloacae* harbouring NDM-1 was contaminated in an imported fishery product. These products contribute to the spread of ARB across countries.

Disclosure: No significant relationships.

Keywords: Carbapenem-resistant bacteria, OXA-48, KPC, NDM, Vietnamese fish

SHIFT 02-042

Topic: ***AS04. Antiviral immunity***

EVALUATION OF SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME VACCINE CANDIDATES IN DOGS

Lecture Title:

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Background and Aims: Severe fever with thrombocytopenia syndrome (SFTS) is a highly fatal disease. The causative agent of the disease, *Dabie bandavirus*, is commonly known as SFTS virus (SFTSV). While the transmission of SFTSV from vertebrates to humans remains ambiguous, there exists verifying evidence implicating dogs as potential transmitters of the virus to humans. Therefore, it is crucial to develop preventive measure against SFTSV in dogs.

Methods: In this study, dogs were immunized three times at two-week intervals with formaldehyde-inactivated SFTSV with two types of adjuvants. SFTSV (KCD46) was injected into all dogs two weeks after the final immunization. To assess the protective efficacy of our vaccine against SFTSV, blood samples were collected from all dogs infected with SFTSV at 2, 4, 7, 10, and 14 dpi.

Results: Viral copy numbers showed high titers in control group until 4 days post infection(dpi). Control dogs exhibited viremia from 2 to 4 dpi, and displayed white pulp atrophy in the spleen, along with a high level of terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling assay (TUNEL) positive area. Though, the inactivated SFTSV vaccine groups exhibited rare pathological changes and significantly reduced TUNEL positive areas in the spleen. Moreover, SFTSV viral loads were not detected at any of the tested dpi. The results indicate that both adjuvants can be safely used in combination with an inactivated SFTSV formulation to induce strong neutralizing antibodies.

Conclusions: Our study highlighted the potential of inactivated SFTSV vaccination as an effective strategy for controlling SFTSV in dogs.

Disclosure: No significant relationships.

Keyword: SFTS, Dog, Vaccine

SHIFT 02-043

Topic: ***AS04. Antiviral immunity***

RISE IN BROADLY CROSS-REACTIVE HUMORAL IMMUNITY AGAINST HUMAN BETA-CORONAVIRUSES IN MERS-RECOVERED PATIENTS DURING THE COVID-19 PANDEMIC

Lecture Title:

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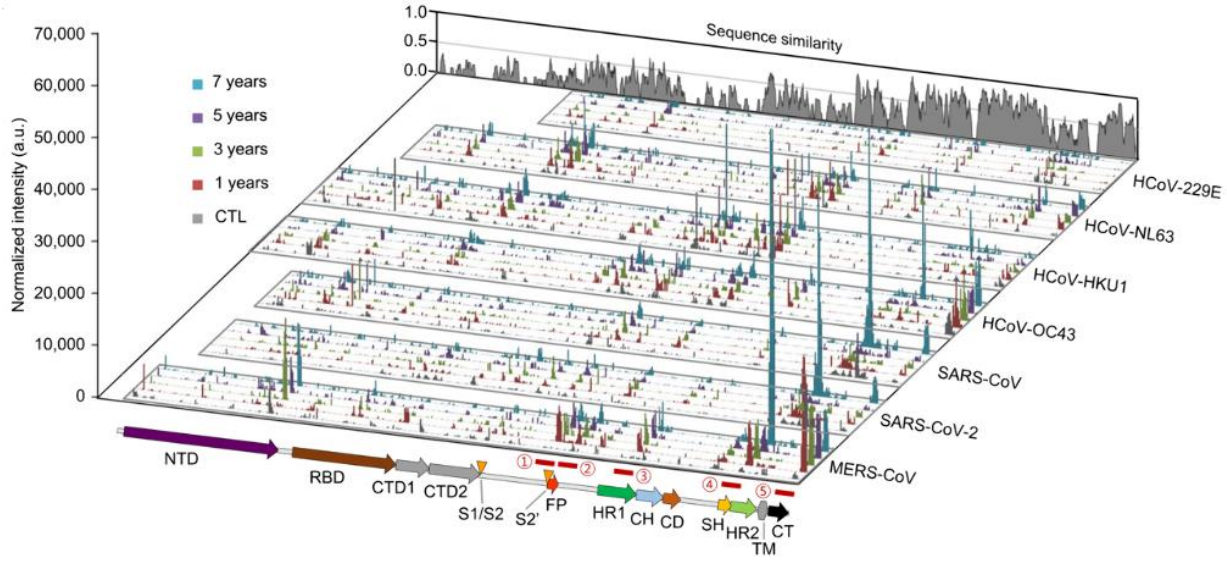
Background and Aims: Developing a universal coronavirus (CoV) vaccine requires the induction of long-lasting, broadly cross-reactive adaptive immunity against various human CoVs and protection against the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants, sporadic Middle East respiratory syndrome (MERS) outbreaks, and future CoV pandemics. Here, we conducted a long-term follow-up study with an aim to elucidate adaptive immune response against MERS- CoV and other human CoVs following the 2015 Korean MERS outbreak.

Methods: ELISA and neutralizing assay were conducted to measure antigen-specific IgG antibody responses and neutralizing antibody levels respectively. Then, peptide microarray system was applied to characterize broadly reactive linear epitopes among 7 hCoVs.

Results: MERS spike(S1)-specific IgG levels and neutralizing antibodies against MERS-CoV declined gradually until the fifth year, with an initial peak in the first 2 years after the MERS outbreak. However, during the coronavirus disease pandemic (the sixth and seventh year), a subset of recovered patients exhibited elevated neutralizing antibody levels against MERS-CoV, possibly due to cross-reactivity with the SARS- CoV-2 vaccine and/or infections. Moreover, The antibody levels significantly correlated across various CoVs, indicating shared immunogenic epitopes. Particularly, two epitopes(stem-helix and cytoplasmic tail domain) were highly immunogenic post-MERS-CoV infection and after SARS-CoV-2 vaccination and/or infection.

Conclusions: Considering the emerging need for a new pan-cov vaccine and our investigation of cross-reactive responses in humoral immunity among various hCoVs, it becomes evident that this

research could be beneficial for efficient vaccine development studies.



Disclosure: No significant relationships.

Keywords: MERS-CoV, COVID-19, SARS-CoV-2, Pan-CoV Vaccine, cross-reactive response in humoral immunity

SHIFT 02-044

Topic: ***AS04. Antiviral immunity***

DELIVERY OF SARS-COV-2 SPIKE AND MEMBRANE GENES IN A SINGLE BACULOVIRAL VECTOR ENHANCE THE IMMUNE BREADTH AGAINST SARS-COV-2 VOCS.

Lecture Title:

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Background and Aims: Even though the coronavirus pandemic is over, the emergence and threat of new variants continues. We constructed three recombinant baculoviral vectored vaccines (AcHERV-COVID19s) that carrying the SARS-CoV-2 prototype, delta, and omicron BA.1 spike gene and confirmed the immunogenicity and cross-protection against SARS-CoV-2 variants. As a vaccine antigen against multiple VOCs, we found that one SARS-CoV2 spike gene alone could not provide protection against multiple VOCs, and that cellular immunity responded more appropriately to different strains.

Methods: To develop a universal vaccine, a recombinant baculoviral vectored vaccine (AcHERV-PanCoV) was constructed by introducing the M gene, which is conserved among VOCs, as a secondary cellular immune antigen in addition to the S gene.

Results: Compared to previously developed vaccines that deliver only the Spike gene (AcHERV-COVID19s), a newly developed AcHERV-PanCoV that simultaneously delivers the S and M genes showed higher protection against SARS-CoV-2 variants (Prototype, Delta, BA.5 and XBB.1).

Conclusions: The membrane protein of SARS-CoV2 has been shown to synergize with the S gene in terms of humoral immunity as well as broad cellular immunity against VOCs. Although AcHERV-PanCoV may not provide sterile protection against the emergence of new variants, it is expected to play a sufficient role in reducing symptoms and stopping the spread of the virus.

Disclosure: No significant relationships.

Keywords: Vaccine, SARS-CoV2, Immune, Broad protection, Membrane Gene

SHIFT 02-045

Topic: ***AS04. Antiviral immunity***

SPECIFIC LONG TERM CHANGES IN ANTI-SARS-COV-2 IGG MODIFICATIONS AND ANTIBODY FUNCTIONS FOR MRNA, ADENOVECTOR AND PROTEIN SUBUNIT VACCINES

Lecture Title:

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Background and Aims: The emergence of SARS-CoV-2 led to rapid COVID-19 vaccine development, primarily inducing IgG antibodies targeting the spike protein. Various platforms allowed immune response comparison. Neutralizing antibodies prevent viral entry .IgG's Fc domain activates innate immunity, crucial for protection. Studies Fc-mediated functions in COVID-19 immunity. IgG efficacy depends on subclass and glycosylation. Long-term changes post-mRNA vaccination are studied, less known for protein subunit vaccines. Taiwan's protein subunit vaccine prompted our study, examining IgG subclasses and Fc-glycosylation, long-term responses, vital for vaccine optimization.

Methods: Samples were collected from vaccinated individuals and pre-vaccine controls. IgG neutralizing titers were determined by ELISA. IgG subclasses were analyzed via Spike protein ELISA. Spike protein-specific IgG was purified and analyzed using mass spectrometry. LC-MS/MS preparation involved sample processing. Accurate inclusion mass screening (AIMS) was conducted using HPLC coupled with a mass spectrometer. THP-1 cells were maintained for ADCP assays. ADCP and ACDC assays were adapted from previous studies and analyzed via flow cytometry. Statistical analysis was performed using R software.

Results: In a cohort of 272 plasma samples from 157 vaccinated individuals, significant changes were observed in antibody responses and functions. Neutralizing titers increased with consecutive doses for most vaccines, while IgG subclass levels rose post-vaccination. Fc-glycosylation remained stable over time, with distinct vaccine responses noted. Limited effects were observed concerning COVID-19 infection, age, and time interval post-vaccination.

Conclusions: Long-term IgG profile changes in three COVID-19 vaccines after three doses and heterologous vaccination with protein subunit and mRNA vaccines. Comparing vaccine platforms in designing optimal immunization strategies for desired immune responses.

Disclosure: No significant relationships.

Keywords: IgG, COVID, Vaccine, isotype (subclass), glycosylation

SHIFT 02-046

Topic: ***AS04. Antiviral immunity***

LONG-LASTING HUMORAL AND CELLULAR MEMORY IMMUNITY TO VACCINIA VIRUS TIANTAN PROVIDES PRE-EXISTING IMMUNITY AGAINST MPOX VIRUS IN CHINESE POPULATION

Lecture Title:

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Background and Aims: In China, Chongqing reported the first imported mpox case in September 2022. Vaccines based on different vaccinia virus (VACV) clades have been developed to achieve smallpox eradication campaign, and Tiantan strain (VTT) used in China. Investigating immune memory to VACV and pre-existing immunity to MPXV is crucial for the global response to this ongoing mpox epidemic.

Methods: We recruited and sampled from 60 individuals inoculated with VTT born before 1981 and 60 unvaccinated controls born since 1982 from Beijing residents. VTT-specific NAb was assessed and cross-NAb against MPXV was tested. Also, antigen-specific T cells were tested by the peptide pools derived from corresponding proteins derived from VTT and MPXV.

Results: 60% or 5% VTT vaccinees possess NAbs to VTT or MPXV, with at least 50% having T cell memory to VTT protein antigens. The eldest with a high level was born in 1943. Among the unvaccinated population, 8.3% have the detectable NAb to VTT, and with 31.7% having the T cell reactivity to VTT. The proportions who had the pre-existing T cell responss to MPXV were 46.7% and 40%. Broad pre-existing $CD8^+$ T cell reactivities to MPXV are detected against conserved and variant epitopes between VTT and MPXV.

Conclusions: In this study, the long duration of the NAb level against VTT was confirmed among the vaccinees. Notably, the pre-existing T cell responses to MPXV were not only detected within VTT vaccinees but could also be found among unvaccinated donors. Additionally, identifying T cell epitopes derived from MPXV in infected individuals contributes to vaccine development.

Disclosure: No significant relationships.

Keywords: Vaccinia Virus Tiantan Strain (VTT), Monkeypox (MPOX), T cell, Neutralizing Antibody (NAb), Pre-exis

SHIFT 02-047

Topic: ***AS04. Antiviral immunity***

ADAPTING LIBRA-SEQ/BEAM TO WHOLE BK POLYOMAVIRUS PARTICLES TO IDENTIFY BROADLY-SPECIFIC HUMAN MONOCLONAL ANTIBODIES.

Lecture Title:

Sarah Marchand[1,2], Manon Loirat[2], Laurence Delbos[2], Martin Braud[2], Lucas Brusselle[2], Cynthia Fourgeux[2], Agathe Trochel[3], Laetitia Gautreau-Rolland[3], Xavier Saulquin[3], Jérémie Poschmann[2], Gilles Blancho[2], Céline Bressollette-Bodin[1,2], Dorian Mcilroy[2]
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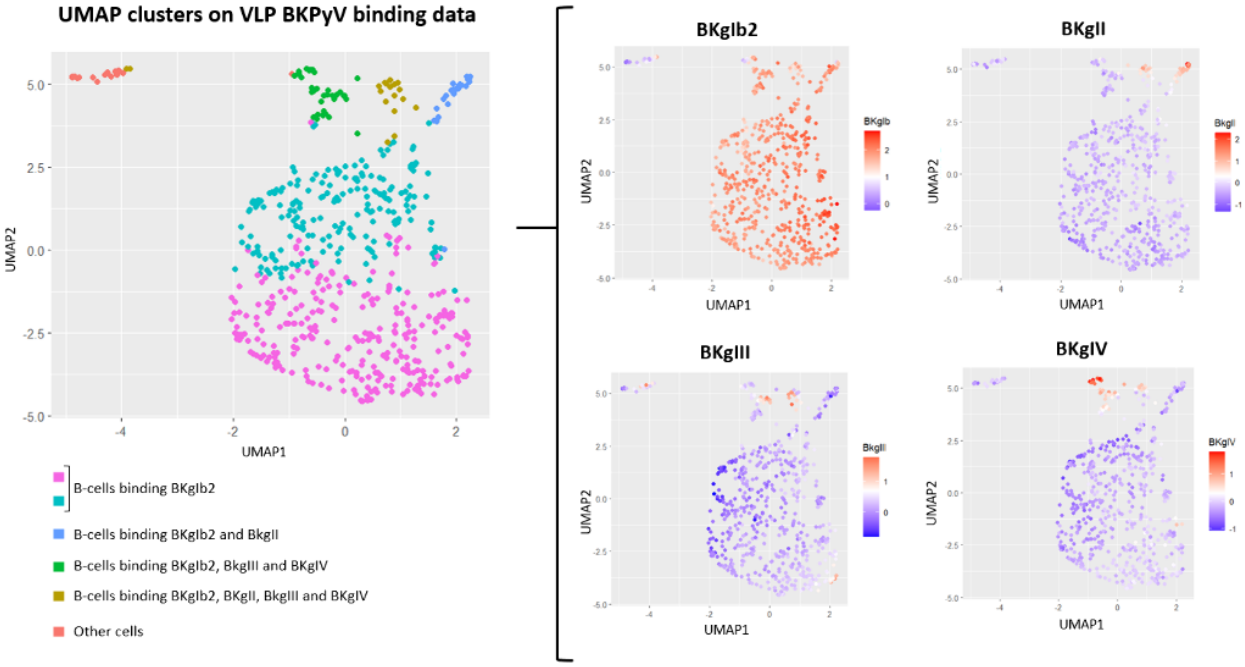
Background and Aims: Data on B-cell repertoires against naked viruses, in particular against BK Polyomavirus (PyV) is relatively sparse. Our objective was therefore to develop and validate a double fluorescence and oligonucleotide labeling of virus like particles (VLPs), in order to apply the LIBRA-seq/BEAM approach to these viruses.

Methods: Fluorescence- and oligonucleotide-labeled VLPs were produced in mammalian cells, using a streptavidin-biotin binding system based on 10X genomics BEAM reagents. VLPs were characterised by SDS-PAGE and negative contrast electron microscopy, and their antigenic and receptor-binding properties were validated by ELISA, and flow cytometry on 293TT cells and PBMCs. A 10X Genomics single cell RNA sequencing experiment on PBMCs from four kidney transplants (KTx) recipients, stained with double-labeled BK (genotypes gIb2, gII, gIII, gIV) and murine PyV VLPs was performed.

Results: The antigenic properties of VLP-streptavidin-oligonucleotide complexes were conserved, as were their binding properties to viral receptors on 293TT cells. Paired light and heavy chain BKPyV-specific BCR sequences were obtained for 588 cells. Overall, 82,3% of B-cells bound only BKPyV gIb2, 4,8% bound gIb2 and gII, 4,3% bound gIb2 and gIV, 1,7% bound gIb2, gIII and gIV, and 3,4% bound all four BKPyV

genotypes.



Conclusions: To conclude, we validated that complete VLPs, and not just individual virus proteins, can be double-labeled with fluorophores and oligonucleotides and used in LIBRA-seq/BEAM. This technique can be used to directly identify broadly binding antibodies within repertoires, and to compare functional repertoire breadth between individuals.

Disclosure: No significant relationships.

Keywords: B-cell repertoires, BK polyomavirus, VLPs

SHIFT 02-048

Topic: ***AS04. Antiviral immunity***

IMPROVEMENT OF THE NEUTRALIZATION ANTIBODY ASSAY FOR EPIZOOTIC HEMORRHAGIC DISEASE VIRUS USING PLASMID-BASED REVERSE GENETICS SYSTEM

Lecture Title:

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Background and Aims: Epizootic hemorrhagic disease virus (EHDV), which belongs to the *Orbivirus* genus of the *Sedoreoviridae* family, represents an ongoing threat to livestock in the world. However, there are still no efficient anti-viral countermeasure such as anti-EHDV drugs and vaccines. Especially, to diagnose EHDV-infection, the current neutralizing antibody test, determined by cytopathic effect (CPE), is sometimes unreliable as the state of samples and serotypes affect the development of CPE. Therefore, in this study, we tried to improve the neutralizing antibody assay method using the reverse genetic system for EHDV and fluorescent gene and evaluated the improved method using blood of beef cattle (Japanese Black) infected with EHDV.

Methods: The series of reassortant EHDV was generated based on EHDV serotype 2 (EHDV-2), strain Ibaraki, back-born and characterized their replication abilities. The segments coding outer capsid proteins (S2 and S6) and other segments replaced with those from EHDV-6 or EHDV-7. The green fluorescent protein (UnaG) gene was induced into segment 9. Heparinized blood samples were collected from Kobe University Farm-raised cattle (adult cattle and calf) and used for the neutralizing antibody assay.

Results: Using our UnaG-labeled reassortant EHDV, we could measure neutralizing antibody titers more precisely. In addition, anti-EHDV antibody was detected in new-born calves from cows with history of EHDV infection.

Conclusions: The calves can be protected from EHDV infection by maternal immunity. Further details regarding neutralizing antibody assay and maternal antibody as well as the characterization of reassortant EHDV will be discussed.

Disclosure: No significant relationships.

Keywords: neutralization antibody, epizootic hemorrhagic disease virus, Japanese Black, fluorescent protein, dsRNA virus

SHIFT 02-049

Topic: ***AS05. Bacteria and climate change***

THE ROLE OF THE PHYLLOSPHERE MICROBIOTA IN THE REGULATION OF TREE-MEDIATED METHANE EMISSIONS IN FLOODPLAINS

Lecture Title:

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Background and Aims: When compared to other land uses, forests have distinct soil properties that modulate microbial communities, including the methanogens and methanotrophs involved in methane production and oxidation. While it is well known that trees can transport soil methane and release it into the atmosphere, the role of methanogens and methanotrophs inhabiting the tree phyllosphere in modulating tree methane fluxes remains relatively unexplored. The aim of this study is therefore to investigate the links between tree methane fluxes, tree traits, and the phyllosphere microbiome for various tree species growing in floodplains, which are known as methane emission hotspots.

Methods: Methanotrophs and methanogens were identified trough 16S rRNA gene sequencing on DNA extracts from wood, bark, and leaves samples collected from five tree species (*Fraxinus nigra*, *Acer saccharinum*, *Populus tremuloides, Salix nigra, Ulmus americana*) in the floodplain of Lake St-Pierre (Quebec). Methane fluxes from tree stems and leaves as well as various tree traits (e.g. pH, density) were also measured.

Results: Our results showed that tree stems can capture or emit methane depending on soil humidity and tree species. We identified methanotrophs and methanogens in the tree phyllosphere, which differed between tree compartments and species according to different traits. Bark methanotrophic communities were correlated with pH and density, and wood methanogenic communities were correlated with humidity. Stem methane fluxes were negatively correlated with the relative abundance of methanotrophs in wood and with the wood density.

Conclusions: Overall, this study will provide a better understanding of the role of the tree phyllosphere microbiota in methane cycling.

Disclosure: No significant relationships.

Keywords: Tree-mediated methane fluxes, Wetlands, Tree microbiome, Climate regulation, Methanotrophs and methanogens

SHIFT 02-050

Topic: ***AS06. Bacterial pathogenicity and virulence factors***

ENTEROCOCCUS FAECALIS VIRULENCE DETERMINES INTESTINAL BARRIER TRANSLOCATION IN MURINE SCLEROSING CHOLANGITIS

Lecture Title:

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Background and Aims: Primary Sclerosing Cholangitis (PSC) is a disease characterized by bile duct inflammation and scarring. Bile ducts can become colonized by bacteria, and we showed that positive bile cultures for *Enterococcus sp.* associated with transplantation-free survival in PSC. Given the unexplored role of biliary colonization and its potential influence on biliary immunoregulation, we aimed to investigate *E. faecalis* colonization dynamics in PSC.

Methods: Bile and stool from people with PSC and controls were collected. *E. faecalis* isolates were retrieved and sequenced, and virulence genes annotated (VFDB). For functional assays, we challenged H69 human cholangiocytes with stool-derived *E. faecalis* lysates, and measured Interleukin-6 concentration by ELISA. We gavaged selected isolates to antibiotic pre-treated mice affected by sclerosing cholangitis (Mdr2-/-) and assessed bacterial translocation to the mesenteric lymph nodes and liver. Liver injury was evaluated by histology, gene expression and serum enzymes measurement.

Results: Genomic analysis highlighted diverse patterns of virulence in bile and stool *E. faecalis*. The presence of the genes *esp* and *gelE* positively correlated with IL-6 production *in vitro*. Virulent *E. faecalis* translocated to the mesenteric lymph nodes of Mdr2-/- mice more frequently than non-virulent *E. faecalis* and induced significantly higher expression of pro-apoptotic *Casp8* in the liver.

Conclusions: *E. faecalis* virulence genes determine its inflammatory potential *in vitro* and its ability to cross the intestinal barrier in Mdr2-/- mice. These results support the notion that *E. faecalis* virulence could be relevant in PSC disease course. Further work is needed to assess whether these findings translate into the context of human bile ducts.

Disclosure: No significant relationships.

Keywords: liver disease, enterococcus faecalis, Pathobiont, cholangitis, gut liver axis

SHIFT 02-051

Topic: ***AS06. Bacterial pathogenicity and virulence factors***

VESICLE PRODUCTION OF PHENOTYPICALLY DIVERSE CLINICAL STRAINS OF PSEUDOMONAS AERUGINOSA

Lecture Title:

Tania Henriquez[1], Francesco Santoro[2], Donata Medaglini[3], Luccia Pallecchi[2], Ilaria Clemente[1], Claudia Bonechi[1], Agnese Magnani[1], Eugenio Paccagnini[1], Mariangela Gentile[1], Pietro Lupetti[1], Massimiliano Marvasi[4], Alessandro Pini[1], Luisa Bracci[1], Chiara Falciani[1]
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Background and Aims: *Pseudomonas aeruginosa* is capable of causing diseases ranging from mild to life-threatening. In recent decades, extracellular vesicles (EVs) have emerged as significant players in the pathogenesis of this organism, facilitating the transport of various molecules. However, it is unknown whether the widely reported phenotypic variability of *P. aeruginosa* clinical strains also affects vesicle production under certain conditions. Here, we analyze the phenotypic diversity of a group of clinical strains of *P. aeruginosa* in relation to vesicle production in minimal medium.

Methods: Fifteen strains of *P. aeruginosa* were analyzed (8 strains collected from clinical samples, 5 from cystic fibrosis patients and 2 reference strains). The phenotypic diversity of the isolates was analyzed through pigment/siderophore production, hemolysis, growth phenotype, antibiotic susceptibility, among others. All strains were grown in minimal medium and used for vesicle purification using a kit.

Results: Our analysis indicated that the clinical strains represent a phenotypically diverse group of organisms. Also, 4 strains were unable to grow on minimal medium, suggesting the possibility of auxotrophies or a phenotype characterized by exceptionally slow growth. Our results indicated that it was possible to isolate vesicles from 3 clinical strains and 2 reference strains, suggesting that the remaining organisms either do not produce vesicles in our conditions (or in negligible amounts) or that purification is affected by factors that favor their elimination (such as the presence of aggregates).

Conclusions: These results corroborate the phenotypic variability of clinical strains of *P. aeruginosa* and suggest that the vesicle production/properties of these strains under given conditions is also diverse.

Disclosure: No significant relationships.

Keywords: Pseudomonas aeruginosa, extracellular vesicles, Phenotypic diversity, Clinical strains

SHIFT 02-052

Topic: ***AS06. Bacterial pathogenicity and virulence factors***

EPIDEMIOLOGY AND VIRULENCE OF EXTRAINTESTINAL PATHOGENIC ESCHERICHIA COLI FROM CAMEL CARCASSES

Lecture Title:

Matěj Hrala[1], Marina Joseph[2], Martina Floriánová[3], Helena Juřicová[3], Ulrich Wernery[2], David Šmajs[1], Juraj Bosák[1]
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Background and Aims: Extraintestinal pathogenic *Escherichia coli* (ExPEC) causes economic losses in livestock globally. However, research on their role in camelid infections remains limited. This study aimed to characterize ExPEC isolates from the world biggest camel farm located in the United Arab Emirates and to identify potential sources of infection.

Methods: A total of 150 extraintestinal *E. coli* isolates were obtained from camel carcasses (isolated between 2004 and 2019) and were analyzed for phylogenetic grouping, serotyping (n=162), and virulence-associated gene (VAG) profiling (n=35) using PCR. Additionally, 139 fecal *E. coli* isolates from healthy adult camels were examined in the same way to assess their role as reservoir of infection.

Results: ExPEC isolates predominantly belonged to phylogroup B1 (58.7%) and exhibited a diverse serotype distribution (n=36). They frequently harbored multiple VAGs associated with adhesion, iron acquisition, cytotoxicity, invasion, and bacterial protection. Moreover, ExPEC profiles differed significantly between adult and calf camels. ExPEC isolates from calves were associated with phylogroups C and E, serogroups O102 and O78, and several VAGs (including *eitA*, *fepC*, *fyuA*, *hlyF*, *iroN*, *iss*, *ompT* and *sitA*).

Conclusions: The identified characteristics of fecal *E. coli* isolates were different from camel ExPEC, suggesting an exogenous source of ExPEC infections, likely transmitted from wild birds and human keepers. *The work was funded by the National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103, Funded by the European Union - Next Generation EU) to DS.*

Disclosure: No significant relationships.

SHIFT 02-053

Topic: ***AS06. Bacterial pathogenicity and virulence factors***

ASSESSMENT OF ARCOBACTER FAECIS AND ARCOBACTER LANTHIERI PATHOGENICITY USING ADHESION, INVASION AND IN VIVO-RELATED FITNESS ASSAYS

Lecture Title:

Izhar Khan[1], Danielle Schneiderman[1], Crystal Ma[2], Wen Chen[3], Wangxue Chen[2], Sebastien Crepin[2]
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Background and Aims: *Arcobacter faecis* and *A. lanthieri,* isolated from human and livestock fecal sources, formed a monophyletic clade and close genetic relationships with human-associated *Arcobacter* species (*A. butzleri, A. cryaerophilus,* and *A. skirrowii*). Our comparative genomics-based study identified several genes associated with adhesion to, and invasion of, host cells. In this study, *in vitro* and *in vivo*-associated virulence assays were performed to investigate the degree of pathogenicity of these species using reference and field isolates for comparative analysis.

Methods: The Caco-2 intestinal cells were used for adhesion and invasion assays. *In vivo*-associated virulence assays such as resistance to oxidative stress (H_2O_2), to cationic antimicrobial peptide (polymyxin B), and to the bactericidal activity of serum were also performed.

Results: Our results showed that *A. lanthieri* cells were significantly more adherent and invasive to eukaryotic cells than *A. faecis* and *A. butzleri*. Similarly, we observed that *A. lanthieri* was more resistant to polymyxin B (cationic antimicrobial peptide), oxidative stress and human serum than *A. faecis*. Moreover, *A. butzleri* and *E. coli,* used as control, also showed similar results.

Conclusions: Together, our results indicate that *A. lanthieri*, compared to *A. faecis*, is potentially pathogenic and can cause health risks to humans and animals. Further *in vivo* study is warranted using models to unravel the mechanism that may be involved in triggering infection by this species.

Disclosure: No significant relationships.

Keywords: Arcobacter faecis, Arcobacter lanthieri, Invasion, adhesion, fitness factors

SHIFT 02-054

Topic: ***AS06. Bacterial pathogenicity and virulence factors***

WHOLE GENOME SEQUENCING ANALYSIS OF ENTEROPATHOGENIC ESCHERICHIA COLI FROM HUMAN AND COMPANION ANIMALS IN KOREA

Lecture Title:

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Background and Aims: Enteropathogenic *Escherichia coli* (EPEC) causes diarrhea, and known to be a major contributor of gastrointestinal disease. In this study, we sequenced 26 EPEC isolates from diarrheic patients and 20 isolates from companion animals in Korea.

Methods: Bacterial genomic DNA was sequenced by Illumina HiSeq (Illumina Inc., San Diego, CA, USA) and Oxford Nanopore MinION (Oxford Nanopore Technologies, Oxford, UK) gene sequencing platforms.

Results: Most isolates were atypical EPEC which do not harboring *bfpA gene*. The most prevalent virulence traits were *ompT* (human: 61.5%, companion animal: 60.0%) followed by lpfA (human: 46.2%, companion animal: 60.0%). Although the pan-genome analyses showed that there was no apparent correlation between the origin of strains, virulence profiles, and antimicrobial resistance profiles however, isolates included in specific clades from human and companion animals had high similarity. Also, all isolates included in this clade encoded *ompT* gene and didn't encode *hlyE* gene. Three typical EPEC strains which encoding *bfpA* were consisted of one isolate from human, and two isolates from companion animals. Those two isolates from companion animals harbored incomplete bundle-forming pilus region which encoding *bfpA* and *bfpB* however, no other *bfp* genes. Interestingly, type IV secretion system associated genes, *tra* and *trb* genes were found in *bfpA* encoding isolate from human.

Conclusions: We conducted a genomic epidemiological analysis of EPEC isolates from diarrheal patients and companion animals. Whole-genome sequencing in this study makes it possible to more accurately analyze the phylogenetic structure of EPEC and provides better insights for the understanding of EPEC epidemiology and pathogenicity.

Disclosure: No significant relationships.

Keywords: Enteropathogenic Escherichia coli, whole-genome sequencing, Pan-genome analysis, One-Health

SHIFT 02-055

Topic: ***AS06. Bacterial pathogenicity and virulence factors***

THE ROLE OF HHA IN VIRULENCE AND COPPER SUSCEPTIBILITY OF UROPATHOGENIC PROTEUS MIRABILIS

Lecture Title:

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Background and Aims: Copper is a component of human innate immune arsenal for fighting invading pathogens. Increasing evidences support that copper is involved in microbial pathogenesis and Cu resistance is crucial for virulence of many bacteria. *Proteus mirabilis* is a leading cause of the urinary tract infections (UTIs). We investigated the Cu resistance of *P. mirabilis* and its effect on virulence.

Methods: We isolated copper-susceptible mutants of *P. mirabilis* by transposon mutagenesis, analyzed the disrupted genes and characterized their roles in virulence. Virulence factor expression and in vivo virulence were monitored. The reporter assay, qPCR and EMSA were performed to reveal the *hha*-regulated gene expression or Cu-mediated *hha* expression.

Results: We isolated the *hha* mutant exhibiting increased copper sensitivity. *hha* gene encodes a toxin of the TomB-Hha toxin-antitoxin (TA) system. We found that Hha-TomB regulated virulence factors such as motility, biofilm formation, urease activity and stress tolerance. Overdose of Hha resulted in a swimming defect and TomB expression can fix the effect of Hha overdose.
The *hha* mutant displayed reduced virulence in the wax moth model. Hha affected *flhDC* and *pmpA* promoter activities and can bind the *flhDC* promoter DNA fragment. Moreover, copper can increase the *hha* mRNA level.

Conclusions: For the first time, we disclosed the Hha-TomB system-regulated copper susceptibility and virulence in *P. mirabilis*, giving an insight into the roles of *P. mirabilis* Hha-TomB TA system. This study also provides cues for targeting the Hha-TomB system in designing strategies to prevent and treat UTIs caused by *P. mirabilis*.

Disclosure: No significant relationships.

Keywords: Hha, Proteus mirabilis, virulence, copper susceptibility

SHIFT 02-056

Topic: ***AS06. Bacterial pathogenicity and virulence factors***

EVALUATION OF NEW METALLOPROTEINASE INHIBITORS AS A STRATEGY TO DISARM PSEUDOMONAS AERUGINOSA

Lecture Title:

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Background and Aims: A promising strategy to overcome the rising threat of MDR *P. aeruginosa*, is the antivirulence approach that acts by disarming the virulence arsenal of the pathogen. Given the pivotal role of LasB elastase in *P. aeruginosa* pathogenesis, the inactivation of this bacterial multifunctional enzyme seems a promising target in anti-*P. aeruginosa* drug design. This study aimed to assess the ability of newly designed inhibitors of LasB, with an *N*-benzyloxy-aminoacid-hydroxamate scaffold (BAHs), to inhibit the proteolytic activity of recombinant as well as native LasB and to evaluate their cytotoxic potential against eukaryotic cells.

Methods: The ability of BAHs to inhibit LasB and two other extracellular metalloproteinases secreted by *P. aeruginosa* (LasA and alkaline protease) was assessed by standard proteolysis assays.

Results: All the BAHs tested (n= 4) showed similar inhibitory activities against recombinant LasB, with K_i values between 1.7 and 4.5 mM. When tested against *P. aeruginosa* culture supernatants, the most active BAHs showed IC_{50} values between 1.34 to 30.38 mM against LasB. Interestingly, they displayed inhibitory effects also against LasA and the total bacterial proteolytic activity. All the BAHs demonstrated a lack of toxicity against the lung epithelial cell line NCI-H441.

Conclusions: Overall, the results obtained support the potential of the new metalloproteinase inhibitors (BAHs), as an adjunctive strategy in the treatment of *P. aeruginosa* infections. Funding: PNRR THE – Tuscany Health Ecosystem; Spoke 7 - Sub-project 5 - Innovative models for the management of infections caused by antibiotic-resistant bacteria (Project code: ECS00000017; CUP I53C22000780001).

Disclosure: No significant relationships.

Keywords: metalloprotease inhibitors, P. aeruginosa, anti-virulence agents

SHIFT 02-057

Topic: ***AS06. Bacterial pathogenicity and virulence factors***

ASSESSMENT OF THE VIRULENCE POTENTIAL IN HUMAN ISOLATES OF ALIARCOBACTER BUTZLERI

Lecture Title:

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Background and Aims: *Aliarcobacter butzleri* is an emergent enteropathogen, widely spread in environment, humans and animals. Infections by this bacterium in humans are often associated with bacteremia and enteritis. While the pathogenic potential of *A. butzleri* has been recognized, the underlying virulence mechanisms and specific strain features remain poorly understood. Thus, in this work, we aimed to evaluate the phenotypic traits of 25 clinical isolates of *A. butzleri* to assess virulence and variability among strains.

Methods: Antimicrobial resistance to five different classes of antibiotics and to bile salts was determined, followed by evaluation of the susceptibility to oxidative stress. Survival profile to stressful host conditions, such as acidic pH and human serum was also assessed. Finally, motility, biofilm formation and adhesion and invasion of Caco-2 cells was evaluated.

Results: Most of the isolates (16/25) presented multidrug-resistance profiles being resistant to at least three antibiotics. Moreover, all the isolates were resistant to bile salts concentrations found in the human gut. The human isolates showed diverse survival profiles under acidic and serum conditions, although all isolates tolerated pH 4 for at least 20 minutes, and three isolates resisted 60 minutes in the presence of human serum. Five isolates presented low motility and the others moderate or high motility. Concerning biofilm formation, we also found different profiles, with only seven isolates presenting with weak biofilm formation. All the isolates showed adhesion to Caco-2 cells, while just 12 were capable to invade.

Conclusions: In conclusion, phenotypic variability was observed among *A. butzleri* clinical isolates, pointing to strain-specific virulence profile.

Disclosure: No significant relationships.

Keywords: Aliarcobacter butzleri, Clinical isolates, virulence

SHIFT 02-058

Topic: ***AS06. Bacterial pathogenicity and virulence factors***

COMPOSITION OF FATTY ACIDS IN THE MYCOMEMBRANE OF ANTIBIOTIC RESISTANT STRAINS OF M. TUBERCULOSIS

Lecture Title:

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Background and Aims: Tuberculosis is recognized as a global health emergency by the World Health Organization. Constitutive antibiotic resistance in *M. tuberculosis* can be caused by reduced permeability of the cell wall, drug modification, enzymatic degradation, and efflux pumps. The mycobacterial cell wall inherently confers antibiotic resistance, due to its lipid content. Discrepancies have been noted in *M. tuberculosis* between phenotypic resistance and acquired genotypic resistance (mutations in canonical genes). Such discrepancies may arise from variations in the fatty acid composition of the bacterial mycomembrane. This study investigates the fatty acid composition of the mycomembrane and its potential correlation with resistance to first-line drugs.

Methods: Ten clinical isolates of *M. tuberculosis* from México were selected, DNA was extracted and sequenced using Illumina-Miseq. Raw data was analized with FASTQC and TB-Profiler to identify genotypic resistance. Phenotypic resistance was determined using the BACTEC MGIT 960. Phenotypic and genotypic resistance results were compared, and in strains with discrepancies fatty acid determination was performed using Gas Chomatography.

Results: Several inconsistencies arose when comparing genotypic and phenotypic resistance. Gas chromatography analysis revealed that strains with inconsistencies displayed elevated concentrations of methyl esters of fatty acids 16:0, 18:0, 19:0, and tuberculostearic acid, along with reduced concentrations of methyl esters of fatty acid 14:0.

Conclusions: These results suggests that the presence of a higher quantity of fatty acids in the mycomembrane of studied strains exhibiting phenotypic and genotypic inconsistencies in the study strains may directly impact the permeability of the cell wall and explain in part their drug resistance pattern.

Disclosure: No significant relationships.

Keywords: Tuberculosis, mycolic acids, mycomembrane, Resistance

SHIFT 02-059

Topic: ***AS06. Bacterial pathogenicity and virulence factors***

IN SILICO MOBILOME IDENTIFICATION OF SALMONELLA ENTERICA SEROTYPE TYPHIMURIUM STRAINS ISOLATED FROM MEXICO

Lecture Title:

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Background and Aims: *Salmonella enterica* serotype *Typhimurium* is a microorganism of public health relevance. Multilocus sequence typing is a typification method to define the allelic profile or sequence type (ST) utilized for studying the clonal relation in several bacteria. *S. Typhimurium*ST19 is the most common ST globally, but it has been displaced by ST213, identified for the first time in Mexico. The mobilome comprises mobile genetic elements that are closely related to resistance and virulence mechanisms in bacteria. S. *Typhimurium*presents multiple genes associated with resistance and virulence, which can be disseminated through plasmids, transposons, integrons, insertion sequences, and bacteriophages. This study focused on identifying the mobilome associated with *S. Typhimurium* ST19 and ST213 strains isolated from food in Michoacán, Mexico.

Methods: Mobilome identification of 11 strains[SV1] of S. Typhimurium was carried out using bioinformatics tools such as FASTQC, TRIMGALORE, SPADES, ABRICATE (for resistance genes, virulence, and plasmids), ISFINDER, and INTEGRON FINDER.

Results: Our findings reveal a significant distinction between *S.* Typhimurium ST19 and ST213 strains across various genomic and phenotypic aspects. In the ST19 strains, IncFIIB and IncFII plasmids are highlighted, while the ST213 strains exhibit a broader variety of mobile genetic elements, including IncI1, ColRNA, IncA/C2, IncFIB, IncHI2, and RepA. This disparity extends to resistance gene profiles; while ST19 harbors the *aac(6)-Iaa* gene, ST213 strains exhibit a diverse spectrum including *aac(6)-Iaa, flor, tet(A), aph(6), sul2,3, aadA2, oqxA,B, blaCMY-2,* and *blaTEM-1B*

Conclusions: ST213 strains present a higher number of mobile genetic elements compared to ST19 strains, this could represent an advantage for bacteria belonging to ST213.

Disclosure: No significant relationships.

Keywords: Mobilome, Salmonella, sequence type, Resistance, plasmid

SHIFT 02-060

Topic: ***AS06. Bacterial pathogenicity and virulence factors***

GENETIC DETERMINANTS OF ANTIMICROBIAL RESISTANCE IN CLINICAL ISOLATES OF ACINETOBACTER BAUMANNII FROM MICHOACÁN MEXICO

Lecture Title:

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Background and Aims: *Acinetobacter baumannii* is an important etiological agent of infections associated with health care. *A. baumannii* possesses many antibiotic resistance mechanisms. Currently, resistome is used to identify genetic determinants related to antibiotic resistance. These determinants and the clones responsible for infections are related to the geographical origin of the isolates. The aim of this work was to analyze the clonality and genetic determinants responsible for antibiotic resistance in *Acinetobacter baumannii* strains isolated in Mexico.

Methods: Fifty-two *A. baumannii* genomes from Mexico were downloaded from public databases. In addition, 10 strains of *A. baumannii*were selected from nosocomial infections at the "Dr. Miguel Silva" General Hospital in Michoacán, México. Antibiotic susceptibility was determined using VITEK®2 and they underwent DNA extraction and subsequent genome sequencing. Genetic diversity, identification of genetic determinants and mobile genetic elements responsible for antibiotic resistance were evaluated using MSLT, Resfinder and MobileElementFinder.

Results: Five clonal complexes were established, the most frequent was CC2, formed by ST2 and ST1544 The most frequent genetic determinant of resistance was to beta-lactams present in 44 genomes (77.19%), the most frequent being *bla*OXA type genes: 40 (90.9%) and *bla*OXA-66 the most frequent (48.6%). Insertion sequences were present in 87.7% of genomes, the most frequent being IS*Vsa3*: 32 (64%)

Conclusions: The predominant sequence type in Mexico is ST 2 belonging to clonal complex 2. The predominant mechanism of resistance to beta-lactams in Mexico is mediated by blaOXA type genes, in addition to insertion sequences, the most frequent being ISVsa3. The above findings are important for the development of potential antimicrobials that influence this type of resistance.

Disclosure: No significant relationships.

Keywords: A. baumannii, sequence type, Clonal complex, Resistance determinants, insertion sequences

SHIFT 02-061

Topic: ***AS06. Bacterial pathogenicity and virulence factors***

ACINETOBACTER SPP. IN COOKED HAMBURGER: A FOOD SAFETY CONCERN?

Lecture Title:

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Background and Aims: *Acinetobacter* species are commonly found in various environments, including animals and humans, such as *Acinetobacter baumannii*, *A. calcoaceticus* and *A. pitti*, recognised as opportunistic pathogens. The contribution of food and water to human infections by *Acinetobacter* spp. remains unclear, but pathogenic strains often exhibit antibiotic resistance. The presence of these microorganisms in meat raises concerns, particularly in relation to cooking. The aim of this study was to evaluate whether *Acinetobacter* spp. in hamburgers can survive cooking temperatures (45, 60 and >70ºC) and simulated gastrointestinal (GIT) conditions.

Methods: Four *Acinetobacter* spp. isolates (*A. baumannii* 114.3, *A. baumannii* 116.2, *A. calcoaceticus* 109.11, *A. johnsonii* 112.1) from meat were inoculated into hamburgers and stored in refrigeration for 24h prior to cooking (well-cooked >70ºC, medium cooked 60ºC, rare cooked 45ºC). Uncooked samples were used as controls. After cooking, the hamburgers were subjected to simulated GIT conditions to assess isolate survival.

Results: All isolates survived medium and rare cooking with reductions of 0.9 to 1.5 log colony forming units (cfu)/g. Except for *A. calcoaceticus* 109.11, which did not survive GIT simulation, the other isolates showed reductions of 1.6 to 2.6 log cfu/g after GIT simulation for both cooking levels.

Conclusions: These results suggest that the isolates tested were resistant to key foodborne infection barriers such as cooking temperatures and GIT conditions (pH 3-7, bile salts). While further research with more isolates is needed to draw definitive conclusions, this study highlights the potential survival of *Acinetobacter* spp. during food preparation and subsequent digestion after consumption of contaminated products.

Disclosure: No significant relationships.

Keywords: Acinetobacter spp., Food safety, Foodborne pathogen, Gastrointestinal tract

SHIFT 02-062

Topic: ***AS07. Bacterial structural biology***

CHARACTERIZATION OF MECHANISM IN DESEMZIA SP. STRAIN C1 LEADING TO HIGH PRODUCTION OF H2O2

Lecture Title:

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Background and Aims: *Desemzia* sp. strain C1, which exhibits a high hydrogen peroxide (H_2O_2) production capability, was isolated from oil-contaminated soil. Strain C1 could produce 1.6-1.8 mM of H_2O_2 in the presence of 10 mM lactate, which was significantly higher compared to the representative H_2O_2-producing bacteria *Streptococcus oralis* and *Aerococcus viridans* under the same conditions.

Methods: The mechanisms of strain C1 leading to high H_2O_2 production were characterized through proteome and whole-genome analysis. The expression of lactate oxidase and peptide-methionine (R)-S-oxide reductase (*MsrB*) was significantly upregulated, showing a correlation with the amounts of flavin mononucleotide and H_2O_2 production. These enzymes were expressed in *E. coli* to determine their specific roles in H_2O_2 production.

Results: As a result, *MsrB* could restore lactate oxidase by reducing oxidized methionine under oxidative conditions. The homo-tetramer ratio and H_2O_2-producing ability of lactate oxidase increased noticeably after *MsrB* treatments.

Conclusions: In summary, the coupled reaction of *MsrB* and lactate oxidase contributes to the high H2O2 production by *Desemzia* sp. strain C1.

Disclosure: No significant relationships.

Keywords: Desemzia sp. strain C1, Hydrogen peroxide, peptide-methionine (R)-S-oxide reductase, Lactate oxidase

SHIFT 02-063

Topic: ***AS07. Bacterial structural biology***

DISTINCT ROLES OF SHEATH PROTEINS IN FLAGELLAR COILING AND STIFFNESS IN LEPTOSPIRA BIFLEXA

Lecture Title:

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Background and Aims: The flagella of *Leptospira* spp. exhibit a coiled shape when isolated, which is essential for bending the cell ends. Leptospiral flagella are composed of a core filament and a sheath, and the sheath contains at least three proteins, FcpA, FcpB, and FlaA. In this study, we investigated the role of each sheath protein in flagellar formation in *L. biflexa*.

Methods: Sheath protein-deficient and its complemented strains of *L. biflexa* were generated, and their flagella and those isolated from the wild-type strain were analyzed by cryo-electron microscopy.

Results: Cryo-electron microscopy of flagella isolated from the wild-type strain showed the localization of the sheath formed by FcpA and FcpB proteins on the outside of the curved flagella. The asymmetric localization of FcpA in the FcpB-deficient mutant curved the flagella. The absence of FlaA2 affected the asymmetric coat localization of FcpA and FcpB, synthesizing straight flagella isotropically coated by FcpA and FcpB. Although the FcpB-deficient mutant exhibited unbent cell ends, isolated FcpB-deficient flagella maintained curvature to the same extent as wild-type flagella.

Conclusions: This study demonstrates the distinct roles of sheath proteins in flagella formation in *L. biflexa*: FcpA is a major flagellar coiling protein, while FcpB functions in strengthening flagellar rigidity. FlaA2 is an essential protein that controls the localization of FcpA and FcpB and then generates the curved structure of the flagella.

Disclosure: No significant relationships.

Keywords: Leptospira, Leptospirosis, Flagella, Spirochete

SHIFT 02-064

Topic: ***AS08. Biofilms***

ASSESSING THE HEALTH RISKS ASSOCIATED WITH THE USAGE OF WATER-ATOMIZATION SHOWER SYSTEMS IN BUILDINGS

Lecture Title:

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Background and Aims: In the context of climate change policies, buildings must implement solutions to reduce energy and water consumption. One solution is showering with water atomization showerheads, which can significantly reduce water and energy usage. However, the lack of risk assessment for users' health has hindered the widespread adoption of this technology. To address this gap, we assess the risk of spreading bacteria, in particular the pathogenic bacterium *Legionella pneumophila*, from shower hose biofilms of different ages grown under controlled or uncontrolled conditions considering different levels of water hardness, during showering using water atomization showerheads (ECO) or continuous flow showerheads (STA).

Methods: We compared the aerosol and bioaerosol emission during a 10 min shower event between the two shower systems

Results: We showed that water-atomization showerhead emitted slightly more nanoparticles smaller than 0.45 µm and slightly fewer particles larger than 0.5 µm than the continuous flow showerhead. When *Legionella pneumophila* was detected in biofilms, ECO showerheads released slightly less cultivable *Legionella* in the first flush of shower water compared to the STA, ranging from 6.0×10^2 to 1.6×10^4 CFU·L^{-1}. However, cultivable *L. pneumophila* was not detected in the aerosols emitted during showering with either showerhead.

Conclusions: These findings suggest that emerging water-drop emission technologies might affect human exposure to aerosols differently than traditional systems, emphasizing the importance of assessing the health risks associated with any new shower system. Additionally, these findings provide valuable insights for achieving a balance between water and energy conservation.

Disclosure: No significant relationships.

Keyword: Bioaerosols; Shower experiment; Legionella; Atomization technology; Water and energy saving

SHIFT 02-065

Topic: ***AS08. Biofilms***

REDUCTION OF BIOFILM ABUNDANCE AND TAXONOMIC COMPOSITION IN SHOWER SYSTEMS BY WATER VAPOR TREATMENT

Lecture Title:

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Background and Aims: Showering presents a risk of exposure to Legionella pneumophila, which may result in Pontiac fever or Legionnaires' disease. While various methods exist to control Legionella in water tanks, none have been tested for treating the terminal part of the system, the shower system. This study aims to address this gap by exploring the impact of a water/vapor treatment on biofilm in shower systems in order to propose a more durable intervention than the replacement of the hose.

Methods: Shower hose biofilms of varying ages, cultivated under controlled or uncontrolled conditions with different water hardness levels, were subjected to a water/vapor treatment. A comparison was made with untreated biofilms from twin hoses grown under identical conditions. Biofilm composition, abundance, and physiological status were assessed using metabarcoding, culture, electron microscopy, ATP quantification, and flow cytometry techniques.

Results: The results demonstrate a significant reduction in biofilm abundance following a single water/vapor treatment, as evidenced by decreased ATP concentration, total cell counts (TCC), and electron microscopic visualization. The remaining bacteria in the treated biofilms were predominantly identified as Proteobacteria, comprising 99-100% of cells. Although the water/vapor treatment resulted in a notable reduction in Legionella pneumophila abundance by three orders of magnitude, a considerable number of individuals of this bacterium persisted, and they remained cultivable even after treatment.

Conclusions: The use of water-vapor treatment to control biofilms in shower systems has demonstrated promising results. However, the optimal timing for treatment remains to be determined.

Disclosure: No significant relationships.

Keyword: Legionella pneumophila; microbial community; NGS; biofilm

SHIFT 02-066

Topic: ***AS09. Bioremediation***

BIODEGRADATION POTENTIAL OF AIR POLLUTANT BY PHYLLOSPHERE MICROBIOME IN MILAN URBAN AREA

Lecture Title:

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Background and Aims: Plants and their microbiomes represent a new potential in the bioremediation field applied to the degradation of polycyclic aromatic hydrocarbons (PAHs): these compounds derive most from the human activity and tend to accumulate in urban air. Air PAHs adsorbed on the leaves area where epiphytic microbial communities are in contact with these pollutants. It has been already demonstrated that phyllosphere bacteria can biodegrade pollutants, but the factors that affect their selection, the rate of the biodegradation process and the estimation of the contribution of contaminant removal are still unknown. The aim of this project is to better understand the ecological factors that determine the composition of the phyllosphere microbiome on four plants in four different seasons and the relative ecological service, the degradation of PAHs in three different areas of Milan, a urban traffic road, a peri-urban park and a large park outside the city.

Methods: Quantification (qPCR) of 16S rRNA gene for bacteria, ITS gene for fungi and nahAc-narAa genes for catabolic activity against naphthalene were carried out on leaves to evaluate the degradation potential of microbial community. Metagenomic sequencing techniques on 16S rRNA and ITS1 was applied to both leaves and bioaerosol.

Results: The data obtained from the statistical analysis will be compared with the chemical data, or the air concentration of PAHs and particulate matter (PM10), to better understand how the microbes respond to air pollution.

Conclusions: The results of this study can be useful to understand how we can improve the degradative activities to improve the urban air quality.

Disclosure: No significant relationships.

Keywords: Microbiome, phyllosphere, leaves, Pollution, phylloremediation

SHIFT 02-068

Topic: ***AS09. Bioremediation***

ARSENIC REMOVAL USING ARSENITE OXIDIZING BACTERIUM, BOSEA SP. BH3 WITH MAGNETITE-MODIFIED BIOCHAR

Lecture Title:

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Background and Aims: Arsenic (As) is recognized as a toxic element in the environment occurred by geochemical processes and Human activities. Biochar, a porous carbonaceous material, has been widely used as an adsorbent for As remediation. However, adsorption to biochar varied depending on the species of arsenic. This study aimed to isolate a novel arsenite-oxidizing bacterium and to synthesis biochar that adsorbs arsenate oxidized by bacterium.

Methods: Soil sample from mine tailings was used to isolate arsenite oxidizing bacteria. Among the obtained strains, one strain (BH3) had the ability to oxidize arsenite, was identified and its physiological and genomic characteristics were analyzed. Additionally, we monitored adsorption of arsenic using magnetite-modified biochar produced from pyrolysis of coffee grounds.

Results: Strain BH3 could oxidize 2 mM of arsenite to arsenate completely under aerobic condition for 5 days. It was able to oxidize 9 mM arsenite and tolerated the toxicity of 100 mM arsenate. It could grow at 8-40 ℃ and at pH 5.5-10.0. The draft genome was 5.25 Mbp and contained the genes related with arsenite oxidation and arsenic detoxification. Comparative genomic analysis showed that strain BH3 was a novel species in the genus *Bosea*. The maximal adsorption capacity of arsenate oxidized by strain BH3 using biochar (1g/L) was 6.5 ppm. In abiotic control, almost no adsorption occurred for arsenite.

Conclusions: We propose the co-utilization of magnetite-modified biochar with arsenite-oxidizing bacterium to enhance the adsorption capability for arsenic. This approach has the potential to be an environmentally friendly solution strategy for arsenic contamination.

Disclosure: No significant relationships.

Keywords: Arsenic, Bosea, biochar

SHIFT 02-069

Topic: ***AS09. Bioremediation***

METABOLIC STUDIES OF WIDE-RANGE PESTICIDE DEGRADING BACTERIA ISOLATED FROM AGRICULTURAL SOIL

Lecture Title:

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Background and Aims: Recalcitrant character of many pesticides remains in soils and sediments for a long time. Therefore, many pesticides have a possibility of presence in soil and groundwater. Pyrethriod pesticides have been used to control pests and reduce crop loss. Etofenprox and Azoxystrobin are a widely used pesticide in agriculture. Therefore, it is important to find microorganisms that degrade wide range causing pollution in the ecosystem.

Methods: etofenprox and azoxystrobin-degrading bacterium was isolated through enrichment processes from agricultural soils. The degradation pathway was studied by GC-MS analysis. Complete genome sequence of the isolate was determined using PacBio platform. The coding sequences (CDSs) were predicted using the Prokaryotic Genome Annotation Pipeline (PGAP). Additional gene prediction and annotation was carried out by RAST annotation system.

Results: The isolate was able to utilize etofenprox and azoxystrobin as a sole source of carbon and energy. Moreover, YH-1 was capable to degrading the other strobilurin and pyrethroid pesticde. Genomic analysis of YH-1 was perforemd with long-read sequencing technic and genome comprised of 7,267,399bp which was assembled in single contigs. This size of genome was 1Mbps lager then previously reported Mycolicibacterium. This is first reported bacterium that simultaneously degrading the etofenprox and azoxystrobin.

Conclusions: A novel etofenprox and azoxystrobin-degrading bacterial strains could utilize and completely mineralize pesticide. Moreover, both bacteria contains some novel hydrolyze-related genes and functional genes. Therefore, these strains are promising bacterial resource for removal of pesticides and its metabolites from the contaminated environments.

Disclosure: No significant relationships.

Keywords: Bioremediation, pesticide, Etofenprox, Azoxystrobin, Mycolicibacterium

SHIFT 02-070

Topic: ***AS09. Bioremediation***

BIOREMEDIATION POTENTIAL OF BACTERIA ISOLATES FROM OILFIELD

Lecture Title:

Nesrine Lenchi
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Background and Aims: Hydrocarbons and heavy metals are two extremely dangerous pollutants that have a big impact on public health. Bioremediation is a cleaning technique that is becoming more and more popular for getting rid of toxic waste from contaminated locations. Green technology that is both economical and environmentally benign should be used to remove these pollutants.

Methods: To do, these strains were incubated in mineral medium in presence of crude oil and/or Heavy metals at 37°C

Results: In just seven days, strain NL4 was able to break down 80% of crude oil in halophilic and alkaline environments. On the other hand, on a medium containing 50% (v/v) crude, the strain NL3 bacteria broke down 77.9% of the crude oil. It was discovered that these strains can decrease 89% of ten distinct heavy metals and can withstand high concentrations of up to 50 mM. Within a short period of 10 days, the addition of these strains to a water sample from a polluted river causes a significant decrease in the levels of many heavy metals, including Cr, Ni, Fe, and others.

Conclusions: These encouraging findings position these bacteria as excellent candidates for further bioremediation initiatives by suggesting that they might be used in more complicated heavy metal and crude oil contaminated environments.

Disclosure: No significant relationships.

Keywords: Bioremediation, Bacteria, oilfield, Heavy metals, crude oil

SHIFT 02-071

Topic: ***AS09. Bioremediation***

CHARACTERIZATION OF PBAT MULCH FILM DEGRADATION BY PURPUREOCILLIUM LILACINUM BA1S ENHANCED BY ADDITIVES AND ITS INFLUENCE ON SOIL.

Lecture Title:

Chi-Te Liu
National Taiwan University, Institute Of Biotechnology, Taipei, Taiwan

Background and Aims: PBAT is a promising biodegradable material for mulch films used in agriculture. We isolated a promising fungal strain Purpureocillium sp. strain BA1S from farmland soil, showing a high PBAT film degradation rate. It could decompose approximately 15 wt.% of the PBAT films 30 days after inoculation. To enhance the PBAT-degrading capability of BA1S for practical application, this study aimed to augment its effectiveness by introducing additives and elucidating the underlying mechanisms.

Methods: Various treatments involving the addition of different cations and pH adjustments found that the addition of calcium ions at pH 7.5 enabled BA1S to achieve extremely high weight loss of PBAT in 28 days, reaching nearly 50%. Scanning electron microscopy (SEM) analysis revealed the presence of mucilage-like substances on the entangled hyphae of BA1S on PBAT films when calcium ions were added. To further assess the PBAT biodegradation effect of BA1S in the soil, fungal spores were inoculated on PBAT films in autoclaved soil for 28 days.

Results: The weight loss of PBAT showed that the addition of CaCO3 in the BA1S-inoculated soil significantly accelerated the degradation rate of PBAT to nearly 100%. Soil samples collected from farmland with degrading PBAT mulch films exhibited alterations in fungal composition with genus Mortierella spp., which was significantly more abundant in PBAT-degrading soil. Mortierella spp. included species capable of degrading PLA/PHB blend mulch films.

Conclusions: This study suggested the potential application of P. lilacinum BA1S and calcium additives to enhance PBAT degradation and provided new insights into PBAT degradation in soil.

Disclosure: No significant relationships.

Keyword: Mulch, lipolytic enzymes, cutinase, carbon catabolite repression, additive

SHIFT 02-072

Topic: ***AS09. Bioremediation***

A NEW BACTERIAL BREAKTHROUGH IN POLYURETHANE BIODEGRADATION AND ITS POTENTIAL FOR SUSTAINABLE BLUE ECONOMY VALUE

Lecture Title:

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Background and Aims: Plastic pollution is a serious global issue that needs to be addressed using different approaches, including microbial degradation. Multiple studies have focused on its treatment and elimination. This work aims to evaluate the polyurethane (PU)-degrading capacity of the deep-sea bacterium *Stutzerimonas frequens* GOM2 and explore its potential for a circular plastic economy.

Methods: The potential PU degrading activity of the GOM2 strain was determined by agar plate screenings on Impranil, detecting bacterial metabolic activity and growth, using PU as a carbon source. Physicochemical changes in Impranil caused by the bacterium were observed by FTIR and molecular weight distribution (MWD). The disappearance of toxic PU-precursors, and the appearance of intermediate metabolites were determined by GC-MS. The pathogenic potential of the GOM2 strain, and acute toxicity of Impranil and its degradation products were assessed in *Galleria mellonella* larvae and zebra fish embryos, respectively. Analysis of the GOM2 genome was performed to identify possible genes involved in PU biodegradation.

Results: GOM2 was capable of use Impranil as a carbon source. The strain produced changes in chemical groups of Impranil, reduced its MWD and metabolized most of the PU precursors. PU degradation products with important commercial applications in bioplastic and food production, and compounds with biological and pharmaceutical activities of interest were identified. The GOM2 strain diminished the toxicity of Impranil and did not exhibit pathogenicity. Several genes encoding for enzymes associated with plastic degradation were identified in the GOM2 genome.

Conclusions: In conclusion, *S. frequens* GOM2 is a good potential candidate for valorization of PU wastes.

Disclosure: No significant relationships.

Keywords: Bioremediation, Polyurethane biodegradation, marine bacteria, Polyurethane, Gulf of Mexico

SHIFT 02-073

Topic: ***AS09. Bioremediation***

GENOMIC POTENTIAL OF A BACTERIAL CONSORTIUM TO DEGRADE HYDROCARBONS IN COASTAL SANDS IN A MICROCOSM MODEL

Lecture Title:

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Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Mexico

Background and Aims: Oil spills in the oceans are a major problem worldwide, strongly affecting coastal ecosystems. A large diversity of bacterial genera has been identified with the ability to degrade hydrocarbons, bacterial consortia have high biological processing efficiencies because the division of labor reduces the load thanks to diverse individual metabolic capacities. Advances in genomic approaches have allowed us to gain insight into the degradative potential of their ability to degrade pollutants. In this study we analyzed the metabolic potential of six marine strains capable of degrading medium crude oil (0.01%) individually up to 14.44% and in a consortium mixed in the same ratio up to 24.62% in a mixture of sand and seawater in a microcosm model.

Methods: Genomic DNA was extracted and sequenced using the Illumina NextSeq 500 platform. The genomes were assembled using the Discovar Denovo software and annotated with the Bakta software. The ANI test was performed using PyAni software.
The degradative potential was explored using and specific hydrocarbon degradation gene base (HADEG) and classical annotation.

Results: The characterized isolates corresponded to *Enterobacter* sp., *Halomonas sp.,* two *Pseudomonas* sp., and two potentially new strains of *Stutzerimonas* genus. Genes involved in aliphatic degradation via the Finnerty pathway and enzymes involved in degrading key aromatic hydrocarbon intermediates such as protocatechuate, gentisate and catechol were found.

Conclusions: The metabolic potential demonstrating the specialization of the consortium for medium crude oil degradation in a mesocosms system. Funding: CONAHCYT scholarship: 957581, UNAM-PAPIIT IG200223.

Disclosure: No significant relationships.

Keywords: Hydrocarbon degradation, Bacterial degradation, Sand oil degradation

SHIFT 02-074

Topic: ***AS12. Emerging diseases***

STRUCTURE AND ANTIGENICITY OF EMERGING HENIPAVIRUS FUSION GLYCOPROTEINS

Lecture Title:

Ariel Isaacs[1], Yu Shang Low[2], Kyle Macauslane[2], Joy Seitanidou[2], Cassandra Pegg[2], Stacey Cheung[2], Benjamin Liang[2], Connor Scott[2], Michael Landsberg[2,3], Benjamin Schulz[2,3], Keith Chappell[2,3,4], Naphak Modhiran[2], Daniel Watterson[2,3]
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Background and Aims: In August 2022, a novel henipavirus (HNV) named Langya virus (LayV) was isolated from pneumonic patients in China. This virus is closely related to Mòjiāng virus (MojV), and both are divergent from the bat-borne HNVs, Nipah (NiV) and Hendra (HeV) viruses. The spillover of LayV is the first instance of a HNV zoonosis to humans outside of NiV and HeV, highlighting the threat this genus poses to human health. In this work, we aimed to determine the structures of LayV and MojV fusion (F) proteins to inform on vaccine and therapeutic designs.

Methods: To achieve this, we stabilised the F proteins in the prefusion conformation and made use of cryogenic electron microscopy and mass spectrometry to determine the structures and glycan profiles of F.

Results: We determined the first high-resolution structures of MojV and LayV F to < 3.5 Å. We show that despite sequence divergence from NiV, the F proteins adopt an overall similar structure, but are antigenically distinct as they do not react to known antibodies or sera. Glycoproteomic analysis revealed that LayV F is less glycosylated than NiV F, however possesses a glycan that shields a known NiV neutralising epitope. We leverage this information to design a trivalent prefusion F HNV vaccine, and demonstrated broad-reactivity against several emerging HNVs.

Conclusions: These findings explain the distinct antigenic profile of LayV F, despite the extent to which it is otherwise structurally similar to NiV. Our results carry implications for HNV vaccines and therapeutic development, and indicate an antigenic, yet not structural, divergence from prototypical HNVs.

Disclosure: K.J.C. and D.W. are inventors of the 'Molecular Clamp' patent, US 2020/0040042 & PCT/IB2023/053263. The remaining authors declare no competing interests.

Keywords: Paramyxovirus, cryoEM, Vaccine, Henipavirus, Emerging virus

SHIFT 02-075

Topic: ***AS12. Emerging diseases***

THE FIRST ISOLATION OF SHANXI TICK VIRUS 2 ON ALL HUMAN AND PRIMATE CELL LINES ILLUSTRATING ITS POTENTIAL ZOONOTIC RISK

Lecture Title:

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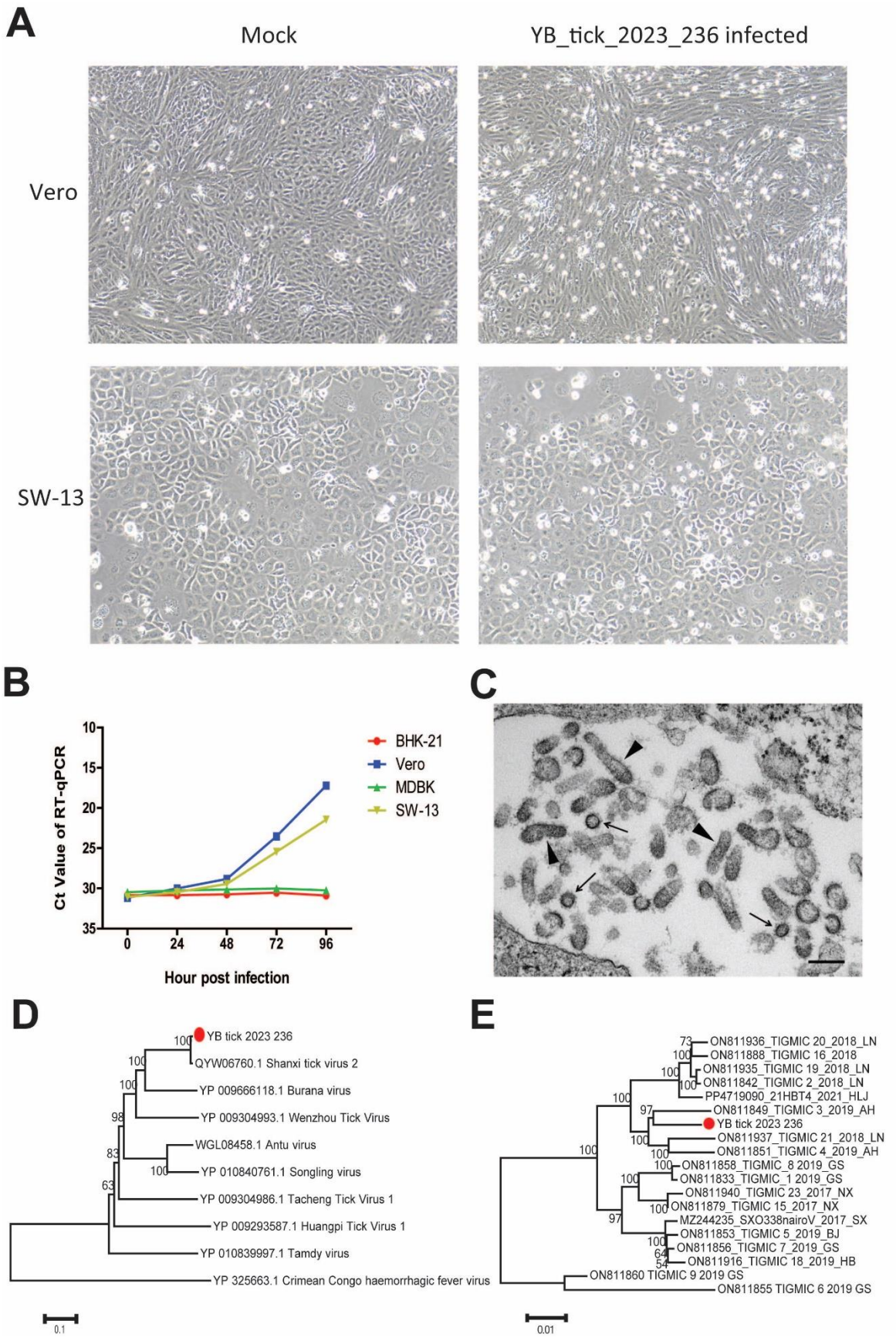
Background and Aims: Shanxi tick virus 2 (SXTV) is a member of the Orthonairovirus genus within the family Nairoviridae. Initially identified through comprehensive genomic sequencing of Haemaphysalis longicornis ticks collected in Shanxi province in 2017.

Methods: We isolated the virus from field-collected ticks using cell culture, identified it through morphological observation and complete genome sequencing, and further characterized it through phylogenetic analysis.

Results:


A
Mock
YB_tick_2023_236 infected
Vero
SW-13
B
Ct Value of RT-qPCR
Hour post infection
BHK-21
Vero
MDBK
SW-13
C
D
YB tick 2023 236
QYW06760.1 Shanxi tick virus 2
YP 009666118.1 Burana virus
YP 009304993.1 Wenzhou Tick Virus
WGL08458.1 Antu virus
YP 010840761.1 Songling virus
YP 009304986.1 Tacheng Tick Virus 1
YP 009293587.1 Huangpi Tick Virus 1
YP 010839997.1 Tamdy virus
YP 325663.1 Crimean Congo haemorrhagic fever virus
E
ON811936_TIGMIC 20_2018_LN
ON811888_TIGMIC 16_2018
ON811935_TIGMIC 19_2018_LN
ON811842_TIGMIC 2_2018_LN
PP4719090_21HBT4_2021_HLJ
ON811849_TIGMIC 3_2019_AH
YB tick 2023 236
ON811937_TIGMIC 21_2018_LN
ON811851_TIGMIC 4_2019_AH
ON811858_TIGMIC 8 2019_GS
ON811833_TIGMIC_1 2019_GS
ON811940_TIGMIC 23_2017_NX
ON811879_TIGMIC 15_2017_NX
MZ244235_SXO338nairoV_2017_SX
ON811853_TIGMIC 5_2019_BJ
ON811856_TIGMIC 7_2019_GS
ON811916_TIGMIC 18_2019_HB
ON811860 TIGMIC 9 2019 GS
ON811855 TIGMIC 6 2019 GS

The

YB_tick_2023_236 strain of the SXTV, derived from Haemaphysalis longicornis ticks collected in the Huichun city of the China-North Korea-Russia border region has been achieved. This isolated strain is capable of replicating and causing cytopathic effects in Vero cells, a primate cell line, as well as in human SW-13 cells, but not BHK-21 and MDBK cells(Figure 1A and 1B). Virus particles were enveloped with spikes, and exhibited pleomorphism with spherical, ovoid, -100 nm in diameter(Figure 1C). Homology analysis comparing YB_tick_2023_236 with SXTV isolate SXO338nairoV showed 95.3% nucleic acid (na) and 99.1% aa identities for the L segment (Figure 1D and 1E). All the terminal nucleotides of L, M and S segments are identical to those of classical orthonairoviruses (3'-segment terminus AGAGUUUCU and 5'-segment terminus AGAAACUCU).

Conclusions: This marks the first isolation of Shanxi Tick Virus 2 (SXTV), underscoring its potential zoonotic risk, as evidenced by its ability to replicate in both human and primate cell lines. Subsequent research should focus on the identification and diagnosis of pathogenic viruses in human clinical specimens and animal samples, as well as on investigating the pathogenicity and mechanisms of the virus using in vitro and in vivo models.

Disclosure: No significant relationships.

Keywords: Shanxi tick virus 2, First Isolation, orthonairovirus, China–Russia-North Korea border

SHIFT 02-076

Topic: ***AS12. Emerging diseases***

IN-SILICO IDENTIFICATION AND BIOCHEMICAL VALIDATION OF NOVEL SARS-COV-2 RDRP NON-NUCLEOSIDE INHIBITORS

Lecture Title:

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Background and Aims: Since its discovery in 2019, SARS-CoV-2 has continued to spread throughout the world, with over 7 million global deaths from the infection. Among the viral non-structural proteins, nsp12 represents the viral RdRp with its cofactors nsp7 and nsp8. To date, only two drugs that target viral enzymes (Remdesivir and Paxlovid) are authorized by EMA for the treatment of COVID-19. The study aims to identify and validate novel SARS-CoV-2 inhibitors through in-silico and biochemical screening.

Methods: Compounds were screened *in silico* against the SARS-CoV-2 nsp12/7/8 complex exploiting the Exscalate platform. Compounds were then assessed according to docking score, novelty on the target based on literature publications and known safety in clinical trials, followed by classification by number and type of docking site. The resulting hit compounds were then screened in a biochemical assay for their capability to inhibit SARS-CoV-2 nsp12 polymerase activity, in presence of cofactors nsp7 and nsp8.

Results: In collaboration with Dompé farmaceutici, two libraries of safe-in-man (>26.000 compounds) and natural compounds (>700.000 compounds) were screened *in silico* against the SARS-CoV-2 nsp12/7/8 exploiting the Exscalate platform by targeting the orthosteric and two allosteric sites of nsp12. Following hit selection, the best 123 hit compounds were then screened in a PAGE-based assay against the viral RdRp activity. Several compounds showed promising inhibitory activity, with IC_{50} values in the low micromolar range.

Conclusions: Several novel non-nucleoside inhibitors of SARS-CoV-2 RdRp were identified by in-silico screening and validated through a biochemical assay. Cell-based assays to evaluate their efficacy on SARS-CoV-2 replication are in progress.

Disclosure: No significant relationships.

Keywords: SARS-CoV-2, nsp12, virology, Antivirals

SHIFT 02-077

Topic: ***AS12. Emerging diseases***

GLANDERS: AN EMERGING ZOONOTIC DISEASE, A CASE REPORT OF HUMAN AND ISOLATION OF AGENT FROM HORSES OF QOM PROVINCE, IRAN

Lecture Title:

Anahita Emadi[1], Shojaat Dashti Pour[2], Mehdi Dehghan Pour[2], Rohollah Keshavarz[2], Nafiseh Shakiba[3], Samerand Reshadi[3], Nader Mosavari[3]
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Background and Aims: Glanders, caused by *Burkholderia mallei*, pose a threat to all solipeds . The bacterium is an ancient zoonotic agent in solipeds and occasionally in humans, especially as an occupational disease in veterinarians and Individuals who work with or handle horses .

Methods: A 22-year-old man was found to have rising Ab titer against *Burkholderia mallei* after a homemade ELISA test. He presented symptoms such as loss of platelet, diarrhea, fever, body aches, cough, and DIC. The initial diagnosis and treatment was for CCHF. The man had a history of close contact with horses. A research study was conducted to isolate and identify *Burkholderia mallei* from the horse farm. At the horse farm first diagnostic tests such as CF, ELISA, western blotting and Malleinisation were performed . A swab and pus culture was taken in the field and transferred to the Glanders diagnostic lab at the Razi Research Institute. The culture was placed on biphasic nutrient agar with 4% glycerin agar and broth medium and incubated at 37 ˚C. DNA was extracted from positive cultures, and IS407-PCR was performed to detect Burkholderia mallei using a specific target.

Results: showed 4 infected horses out of 18 and one isolate growth and identified by PCR.

Conclusions: The reappearance and geographical extension of glanders disease in Iran can be attributed to the international trade of animals, breeding practices, and equestrian sports. In regions where the risk of glanders is high, it is crucial for animal owners and veterinarians to collaborate to control the infection effectively.

Disclosure: No significant relationships.

Keywords: Glanders, ELISA, horses, zoonotic, PCR

SHIFT 02-078

Topic: ***AS12. Emerging diseases***

SEVERITY AND OUTCOME OF LABORATORY CONFIRMED INFLUENZA TYPE AND SUBTYPE IN TERTIARY CARE HOSPITAL OF THE SUB-HIMALAYAN REGION OF INDIA

Lecture Title:

Shailender Negi
All India Institute of Medical Sciences, Rishikesh, Microbiology, Rishikesh, India

Background and Aims: Seasonal variations in influenza virus types and subtypes generate significant variation in the disease load. Despite the availability of vaccinations and antiviral medications, new variations, strains, and subtypes have repeatedly evolved, producing epidemic, zoonotic, and pandemic illnesses, ICU admission, and mortality. Therefore, this study aims to evaluate type and subtype-associated severity and outcome in patients infected with the influenza virus.

Methods: Clinically suspected samples with influenza or influenza-like illness received from different hospital wards from February 2023 to January 2024 were included in the study. A total of 232 nasopharyngeal samples were tested using a Hi-PCR COVID FLU RSV Multiplex Probe PCR Kit to detect influenza A, B, Pandemic H1, and H3 influenza viruses. Mean and standard deviation were reported for continuous variables after checking for normality using the Shapiro-Wilk test and frequency and percentage for categorical variables.

Results: Among 232 cases, 23(9.9%) were positive; of which,13(56.5%) had influenza type A and 10(43.5%) had type B. Among Influenza type A, 7(30.4%) had H1N1, 1(4.3%) had H3N2, 1(4.3%) had H1N1 pdm09 and 4(17.4) were not subtyped. We found hospitalization in all cases 23(100%), ARDS in 15(65.2%), pneumonia in 6(26.1%), mechanical ventilation in 18(78.3%) and death in 9(40.9%). Severe complications and outcomes were reported in Influenza A H1N1 and Influenza B.

Conclusions: Despite high prevalence of influenza type A virus, we found severe outcomes associated with influenza type B virus. Thus, an early antiviral treatment for suspected patients and vaccination for populations at risk against the circulating influenza virus becomes an important public health measure

Disclosure: No significant relationships.

Keywords: influenza, severity, Subtype, disease outcome, mortality

SHIFT 02-079

Topic: ***AS13. Eukaryotic anti-microbial resistance***

META ANALYSIS OF SYNBIOTIC SUPPLEMENTATION AS ALTERNATIVE TO COCCIDIOSTATS AND ANTIBIOTICS IN BROILER CHICKENS: ZOOTECHNICAL EFFECTS

Lecture Title:

Jutta Kesselring[1], Ermioni Papadopoulou[1], Karin Schoendorfer[1], Luis Valenzuela[2], Philippe Tacon[2], Basharat Syed[1], Michaela Mohnl[1]
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Background and Aims: Poultry coccidiosis is an intracellular parasitic infection caused by *Eimeria* spp. that leads to chronic productivity losses, in many situations without causing apparent clinical symptoms. Alternative strategies to antimicrobials are evaluated to manage *Eimeria* infestation in poultry houses. The aim was to systematically synthesize all studies testing a commercial synbiotic product (PoultryStar® dsm-firmenich ANH) in broilers exposed to coccidiosis challenge.

Methods: Data from 2011-2023 feeding trials with broiler chickens, unambiguous description of trial design and animal husbandry conditions, a minimum duration of 42 days, sample size and standard deviation of measurements recorded were eligible. The standardized mean difference SMD, corrected by Hedges' g, was computed. Effect sizes are rated small (SMD 0.2-0.5), medium (SMD 0.5-0.8) and large (SMD >0.8).

Results: Twelve (12) enrolled studies had exposed the birds to a challenge (4 x *Salmonella enterica* Thyphimurium, 9 x *Eimeria* ± *Clostridium perfringens* or *S.* Thyphimurium). Nine (9) enrolled challenge studies tested also an antibiotic (coccidiostat, antimicrobial antibiotic) treatment. The synbiotic preparation significantly improved feed conversion ratio (FCR, SMD=0.5571 , p=0.0065), average daily gain (ADG, SMD= -1.26, p=0.0018) and live weight (BW, SMD= -0.90), p=0.0075) compared to untreated control.

Conclusions: Data collected 2011-2023 suggest that the tested synbiotic preparation is as effective as coccidiostat and/or antimicrobial antibiotic supplementation in preventing poultry productivity losses under challenge conditions. We hope to substantiate these findings with indicators for the mode of action based on the available wealth of data.

Disclosure: All authors are employees of the very same organization that developed, produces and markets the tested synbiotic product for poultry.

Keywords: probiotic, synbiotic, coccidiosis, broiler, meta analysis

SHIFT 02-080

Topic: ***AS13. Eukaryotic anti-microbial resistance***

ANTI-AMEBIASIS DRUG DEVELOPMENT WITH NEW SCAFFOLDS AND MODES OF ACTION

Lecture Title:

Tomoyoshi Nozaki[1], Haruka Ohno[1], Ghulam Jeelani[1], Yuji Sumii[2], Norio Shibata[2], Yumiko Saito-Nakano[3], Natsuki Watanabe[1]
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Background and Aims: Amebiasis is the second largest protozoan killer after malaria, responsible for approximately 1% infection of the population worldwide. Only one class of compounds, nitroimidazoles, are clinically available, but resistance against nitroimidazoles is rising in other metabolically similar bacterial and protozoan pathogens. Thus, new classes of anti-amebic compounds with novel mode of action are necessary.

Methods: We are taking two approaches to develop new anti-amebic compounds: cell-based and target-based screening of chemically elucidated compound library from Drug Discovery Initiative, BINDS. As *Entamoeba histolytica*-specific target, we chose methionine aminopeptidase 2 (MetAP2), which is involved in protein translation/post-translational modification.

Results: Through cell-based screening, we identified several new classes of scaffolds including quinoxalines and purines. We also identified, through MetAP2 inhibitor screening, new classes of MetAP2 inhibitors, which do not share structures with the previously known fumagillin. We established structure-activity relationships for these new classes of inhibitors.

Conclusions: Our cell-based and target-based anti-amebic compound discovery campaign yielded several classes of new inhibitors with a potential to be further developed as anti-amebic drugs

Disclosure: No significant relationships.

Keywords: Entamoeba histolytica, Amebiasis, Methionine aminopeptidase 2, Mechanism of action, Fumagillin

SHIFT 02-081

Topic: ***AS15. Food microbiology***

GENOTYPIC CHARACTERIZATION OF STAPHYLOCOCCUS AUREUS ASSOCIATED WITH FOOD POISONING OUTBREAKS IN JAPAN

Lecture Title:

Shouhei Hirose[1], Ryoji Nomura[2], Rie Doi[3], Miki Sone[4], Tsuyoshi Kudeken[5], Keita Yanagimoto[6], Satoko Yamaya[7], Takuya Mizuno[8], Yuka Kojima[9], Yukiko Hara-Kudo[1]
[1]National Institute of Health Sciences, Kawasaki, Japan, [2]Shimane Prefectural Institute of Public Health and Environmental Science, Matsue, Japan, [3]Saitama Institute of Public Health, Hiki-gun, Japan, [4]Saitama City Institute of Health Science and Research, Saitama, Japan, [5]Okinawa Prefectural Institute of Health and Environment, Uruma, Japan, [6]Yamanashi Institute of Public Health and Environment, Kofu, Japan, [7]Miyagi Prefectural Institute of Public Health and Environment, Sendai, Japan, [8]Gifu Prefectural Research Institute for Health and Environmental Sciences, Kakamigahara, Japan, [9]Kawasaki City Institute for Public Health, Kawasaki, Japan

Background and Aims: *Staphylococcus aureus* remains a major cause of bacterial food poisoning worldwide. *S. aureus* produces staphylococcal enterotoxins (SEs), which are known to cause staphylococcal food poisoning (SFP) in humans. It is important to clarify the genotypic characteristics of *S. aureus* strains associated with SFP. The aim of this study was to analyze the genetic diversity of a panel of *S. aureus* strains associated with SFP in Japan.

Methods: In all, 54 *S. aureus* isolates were assessed in this study. These strains originated from 54 SFP outbreaks collected from 2000 to 2021 across 8 Public Health Institutes in Japan. Genomic DNA of *S. aureus* strains was sequenced on the Illumina MiniSeq instrument. The sequenced reads were trimmed and assembled de novo using the CLC Genomics Workbench with default settings. The Center for Genomic Epidemiology web server was used to conduct MLST analysis. Local BLAST was used to identify enterotoxin genes. Coagulase typing was performed by use of specific antisera.

Results: *S. aureus* strains were divided into 15 clonal complexes (CC), and the most frequent CC type was CC6. More than 60% of the SFP isolates belonged to coagulase types IV and VII. Comparison of the CC types and the SE genotypes showed a dominant genotype of SFP isolates, sea, which exclusively belonged to CC6, CC8, CC88 and CC96, and sea, seb, seh, sek and seq which exclusively belonged to CC1 and CC81.

Conclusions: CC6 was the most frequent CC type of *S. aureus* strains associated with SFP. Dominant SE genotypes were linked to specific CCs.

Disclosure: No significant relationships.

Keywords: Food poisoning, Genotyping, staphylococcus aureus, Staphylococcal enterotoxin

SHIFT 02-082

Topic: ***AS15. Food microbiology***

ASSESSING THE EFFICACY OF UV-C RADIATION VERSUS NON-THERMAL PLASMA IN INACTIVATION OF FOODBORNE FUNGAL SPORES

Lecture Title:

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Background and Aims: Fungal contamination presents an ongoing obstacle across various industries, including food, healthcare, and clinical settings, as fungi demonstrate notable resistance to conventional control methods. Our research focuses on comparing the effectiveness of UV radiation and non-thermal plasma (NTP) against foodborne fungal contaminants (Alternaria alternata, Aspergillus niger, Fusarium culmorum, Fusarium graminearum).

Methods: We investigated the impact of different UV radiation doses on the spores of these fungi while simultaneously assessing the effects of UV radiation and NTP on cell metabolic activity post-germination and their subsequent germination potential.

Results: Our findings indicate that UV-C radiation did not significantly hinder the metabolic activity of cells post-germination. Conversely, NTP demonstrated nearly 100% effectiveness in suppressing both spores and their subsequent germination, with the exception of A. niger. For A. niger, the effectiveness of UV-C and NTP was comparable, resulting in only a 35% decrease in metabolic activity after 48 hours of germination, whereas other strains exhibited reductions of over 95%. Scanning electron microscopy (SEM) images revealed morphological changes in spore structure following both treatments.

Conclusions: This study addresses a critical gap in existing literature by providing insights into the adaptive capabilities of treated cells and emphasizing the importance of exposure duration and nutrient conditions. Our results underscore the promising antimicrobial potential of NTP, particularly against filamentous fungi, suggesting opportunities for enhanced sanitation protocols across various applications.

Disclosure: No significant relationships.

Keywords: UV-C irradiation, NTP, fungal spores

SHIFT 02-083

Topic: ***AS15. Food microbiology***

THE SIGNIFICANCE AND HEALTH IMPLICATIONS OF ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIA ISOLATED FROM LOCALLY MADE SNACKS IN NIGERIA

Lecture Title:

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Background and Aims: In Nigeria, a variety of snacks is widely consumed, especially by youngsters and school children. The increased intake of locally-made snacks requires an examination of their microbial quality and antimicrobial properties, crucial for public health. This research seeks to offer key insights into snack safety, establishing a basis for informed policies in the food industry.

Methods: The study collected 7 diverse samples of traditional snacks bought from different parts of Nigeria. These samples were subjected to microbiological analysis to ascertain the presence of antimicrobial susceptibility patterns. The samples were aseptically blended and serial dilution of up to ten fold were made for the samples. The isolated were identified by conventional methods.

Results: Bacteria were identified from the samples: enterrobacter spp, pseudomonas aeruginosa, klebsiella pneumonia, eschericha coli, micrococcus spp, baccillus spp, streptococcus faecalis and staphyloccucus aereus. The bacterial count on the snacks samples reveals kulikuli and aadun has the most bacteria (6.6x10.7cfu/ml and 5.6 x 10.7) respectively. The antimicrobial susceptibility pattern revealed that pseudomonas aeruginosa had the most resistance to virtually all the antimicrobial agents tested.

Conclusions: This paper shows the presence of antimicrobial susceptibility patterns in locally-made snacks in Nigeria. Many studies have reported that snacks and foods prepared under unhygienic conditions are vulnerable to microbial contamination. There is an urgent need to study the pathogenicity and strain distribution of presumptive food pathogens and how they relate to the hygienic practices of snack preparations in Nigeria. Doing so could disclose the potential of food poisoning epidemics as related to snacks consumption in Nigeria.

Disclosure: No significant relationships.

Keywords: Food, Bacteria, safety, Snacks, Nigeria

SHIFT 02-084

Topic: ***AS15. Food microbiology***

D-PSICOSE, A RARE SUGAR DOWN-REGULATE DEVELOPMENT OF CANDIDA ALBICANS HYPHAE

Lecture Title:

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Background and Aims: D-Psicose ($C_6H_{12}O_6$; D-allulose) is one of famous rare sugar that exists as a monosaccharide in nature. It has been recently reported that D-psicose has a variety of physiological effects as anti-inflammatory and antioxidant effects in animals and humans. In this study, we tested the effect of D-psicose on the growth of *Candida albicans* strain ATCC 10261 (Ca).

Methods: We first checked morphological changes using two microscopic techniques in liquid peptone mediumcontaining 4% D-glucose (Sabouraud's medium; SM) or 4% D-psicose (PM) and performed the MTT assay. Next, we analyzed the expression of virulence-related genes in Ca when cultured in liquid medium containing 4% D-psicose at 37°C under aerobic conditions using quantitative reverse transcription-PCR (qRT-PCR) methods.

Results: Compared to culturing in SM, Ca took longer to reach the stationary growth phase in PM. In the MTT assay, Ca strain ATCC 10261 cultured in liquid PM showed a significant reduction in metabolic activity when compared culturing in liquid SM, suggesting that D-psicose reduces Ca viability. Interestingly, microscopic analysis revealed the inhibition of hyphal development in Ca cultured in liquid PM at 37°C. In addition, qRT-PCR showed downregulation of the expression of virulence-related genes *HWP1* and *PLB1* in Ca cultured in liquid PM compared to Ca cultured in liquid SM.

Conclusions: Taken together, these results show that D-psicose weakens the growth and inhibits hyphal development of Ca, and strongly suggest that D-psicose suppresses Ca morphogenesis and biofilm formation.

Disclosure: No significant relationships.

SHIFT 02-085

Topic: ***AS15. Food microbiology***

ADMINISTRATION OF LACTOCOCCUS LACTIS LB1022 ALLEVIATE ATOPIC DERMATITIS TO ASTHMA THROUGH THE REGULATION OF THE MICROBIOTA-GUT-SKIN-RESPIRATORY TRACT AXIS

Lecture Title:

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Background and Aims: It is well known that probiotics effectively improve atopic dermatitis (AD), but its effects on AD to asthma (atopic march) remain unclear. In this study, we evaluated the efficacies through which probiotic LB1022 inhibited the progression of atopic march and compared these effects with those of prebiotic, probiotic, and postbiotic supplementation in an atopic march mouse model.

Methods: Five-week-old female BALB/c mice were sensitized with ovalbumin, then treated with a synbiotic (fructo-oligosaccharide and *Lactococcus lactis* LB1022), prebiotic (fructo-oligosaccharide), probiotic (*L. lactis* LB1022), and postbiotic (heat-killed *L. lactis* LB1022) for 8 weeks.

Results: The synbiotic dietary supplementation significantly alleviated allergic inflammation and symptoms associated with AD and asthma by regulating Th2/Th1 cytokine imbalance compared to prebiotic, probiotic, and postbiotic supplementation. In particular, synbiotics significantly increased SCFA-producing bacteria such as Lactobacillus and Bifidobacterium spp.

Conclusions: These findings suggest that synbiotics have the potential to be a food supplement for improving AD-linked allergic airway inflammation.

Disclosure: No significant relationships.

Keywords: Atopic march, gut microbiome, Atopic dermatitis, Asthma, Lactococcus lactis

SHIFT 02-086

Topic: ***AS15. Food microbiology***

RELATIONSHIP BETWEEN FLOCCULATION AND ETHANOL ADAPTATION IN THE BREWER'S YEAST SACCHAROMYCES PASTORIANUS

Lecture Title:

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Background and Aims: *Saccharomyces pastorianus*, which has strong flocculating ability, is used in lager beer. Therefore, the objective of this study was to elucidate the effect of *S. pastorianus* flocculation on ethanol adaptation and production.

Methods: The conditions under which *S. pastorianus* TUM 34/70 and the type strain *S. pastorianus* NBRC 11024 flocculate were investigated, focusing on glucose, maltose, ethanol, and $CaCl_2$. Ethanol tolerance tests and measurement of ethanol production were conducted using TUM 34/70 flocculated and TUM 34/70 deflocculated by EDTA. In addition, gene expression levels of genes involved in flocculation (Lg-*FLO1*) and ethanol dehydrogenase gene (*ADH1*) were measured. At that time, we focused on the strength of flocculation and differences in ethanol concentration (0, 5, and 10% ethanol).

Results: TUM 34/70 had the highest flocculating ability in YPD medium with Ca^{2+}; flocculating yeasts survived better in 10% ethanol. When compared by flocculence, the more flocculent yeasts had higher growth rate and ethanol production, and expressed higher levels of Lg-*FLO1* and *ADH1*. The expression of Lg-*FLO1* increased at 5% ethanol concentration, but decreased at 10%. On the other hand, *ADH1* decreased with increasing ethanol concentration.

Conclusions: Yeasts in the center of the flocculence may be less susceptible to ethanol stress because they are protected by the external yeasts. Flocculation may also play a role in the yeast's adaptation to ethanol, as flocculated yeasts were able to maintain high survival rates even after exposure to 10% ethanol.

Disclosure: No significant relationships.

Keywords: brewer's yeast, Saccharomyces pastorianus, flocculation, ethanol, ethanol adaptation

SHIFT 02-087

Topic: ***AS15. Food microbiology***

GENOMIC ADAPTATION OF SALMONELLA IN THE POULTRY FARM: AN ARTIFICIAL INTELLIGENCE APPROACH TO ANTIMICROBIAL RESISTANCE AND STRESS RESISTANCE

Lecture Title:

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Background and Aims: The study of *Salmonella* resistance to different industrial environments is essential to understand its adaptation to various stress factors. In the poultry industry, *Salmonella* enterica stands out for its multi-resistance and virulence. We focused on the adaptation of *Salmonella* isolates to multiple stressors present in a poultry farm.

Methods: Using a machine learning approach, we identified *Salmonella* genome modifications that are characteristic for surviving industrial stress. Thus, we characterized 186 genomes of *Salmonella* isolates. Subsequently, we evaluated the physiological characteristics of the strains in response to oxidative, acid and osmotic stress and antibiotic resistance.

Results: The results show that 75% of the isolates are resistant to the stresses evaluated, and 81% are biofilm formers, quadrupling their resistance. Particularly, isolates of serotype Infantis present in the slaughterhouse showed higher resistance. In addition, with the analysis of promoters and sigma factors, we obtained a classification of markers by objectively searching for patterns in response to environmental stress. Finally, a machine learning system was developed that integrated all the physiological, genomic and transcriptional information identifying the adaptation of *Salmonella* to stress present in the poultry farm, with high precision and accuracy in the classification of isolates.

Conclusions: In conclusion, the importance of understanding *Salmonella* adaptation and resistance in the poultry industry is highlighted. The integration of advanced technologies and artificial intelligence tools holds promise for addressing these challenges. This study aims to establish a predictive capability based on genomic surveillance of *Salmonella* that can be applied together with artificial intelligence with genetic information. Fondecyt 1210633

Disclosure: No significant relationships.

Keywords: Salmonella, Genomics, MACHINE LEARNING, Poultry Farm, Multidrugs Resistance

SHIFT 02-088

Topic: ***AS15. Food microbiology***

AUTOINDUCER-2 SIGNALING AND BIOFILM PRODUCTION IN MEAT SPOILER PSEUDOMONAS FRAGI

Lecture Title:

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Background and Aims: Meat spoilage under refrigerated conditions significantly impacts the economy due to the loss of consumable meat, with *Pseudomonas fragi* being a major contributor through its biofilm production capabilities. This study aims to investigate the role of Quorum Sensing (QS) and the production of autoinducer-2 (AI-2) in the spoilage process, with the goal of identifying strategies to reduce spoilage and associated economic losses.

Methods: Sampling involved collecting 20 meat samples, from which 337 *Pseudomonas* isolates were obtained. Specific primers identified putative *P. fragi* isolates, while rpoD sequencing provided detailed phylogenetic analysis. Biofilm formation was assessed using the tube method, microtiter plate assay, and Congo red agar test. AI-2 production was screened using the luminescence of a Vibrio harveyi strain biosensor.

Results: Out of 83 *Pseudomonas* species isolated, 15 were identified as *P. fragi* and 57 as *P. bubulae*, a closely related species. All isolates showed active motility, and 37 exhibited biofilm production. Among these, 18 demonstrated AI-2 production via luminescence, suggesting a potential QS activity related to spoilage processes.

Conclusions: The findings suggest a correlation between AI-2 production and biofilm formation in *Pseudomonas* species, indicating a complex mechanism of meat spoilage that involves QS. Understanding the role of AI-2 in *P. fragi*-induced spoilage could lead to innovative approaches to mitigate spoilage, potentially reducing the economic losses in the meat industry. Future research will focus on elucidating the precise mechanisms of QS in meat spoilage and exploring intervention strategies.

Disclosure: No significant relationships.

Keywords: Biofilm, meat, Pseudomonas, quorum sensing

SHIFT 02-089

Topic: ***AS15. Food microbiology***

A METATAXONOMIC SURVEY OF THE BACTERIAL MICROBIOTA OF TABLE OLIVES

Lecture Title:

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Background and Aims: Table olives are among most ancients fermented foods. At least 4 main styles (Spanish-style; Picholine; Greek style; California-style) are recognized, but spice addition, stone removal, drying, dry salting cracking and/or addition of spices add furhter variation. Starter addition is possible, but natural contamination and ecological factors drive the microbial community dynamics and affect quality of the finished product. The aim of this work was to characterize the bacterial community of a large variety of table olives using amplicon targeted metagenomics.

Methods: Olives were obtained from producers with their brine. Microbial biomass was recovered by centrifugation and DNA was extracted using PowerFood kit. Sequencing of V3-V4 region of 16S RNA gene was performed using Illumina NovaSeq. Sequences were analyzed using a pipeline based on DADA2 and taxonomic assignment was performed using SILVA 138.1 as reference.

Results: The microbiota of table olives was diverse (>300 taxa identified at genus level). Genera with average relative abundance >0.05 and prevalence >0.25 were Lentilactobacillus, Lactiplantibacillus, Pediococcus, Marinilactibacillus, and Alkalibacterium, but 10 more genera had an average abundance >0.01. Naturally fermented olives had a larger diversity and halophilic and alkaliphic microorganisms were frequent in Spanish style and Picholine olives. Similarity within producers was higher than between producers even for the same variety.

Conclusions: We built a large catalog of the bacterial diversity of table olives. Further data will be added within the framework of the METAolive project and will be combined with thos available in the FoodMicrobionet database, thus providing invaluable information on the microbial ecology of this product.

Disclosure: No significant relationships.

Keywords: table olives, bacterial microbiota, metataxonomic survey

SHIFT 02-092

Topic: ***AS15. Food microbiology***

GENETIC DETERMINANTS ENCODING ARSENIC, CADMIUM, BENZALKONIUM CHLORIDE RESISTANCE IN LISTERIA MONOCYTOGENES ISOLATED FROM FOOD

Lecture Title:

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Background and Aims: *Listeria monocytogenes* is a foodborne pathogen that causes listeriosis. The environmental adaptation of *L. monocytogenes* is a complex process involving heavy metal resistance. **The aim** of this study was to analyze whole-genome sequencing data from a set of 45 strains of *L. monocytogenes* isolated from food in order to compare the prevalence and types of genetic determinants encoding resistance to cadmium, arsenic, and additional benzalkonium chloride.

Methods: The DNA was extracted using the E.Z.N.A.® Bacterial DNA Kit (Omega Bio-Tek, USA). Following whole genome sequencing the presence of the monitored genes was identified utilizing SeqSphere+ Software (Ridom GmbH, Munster, Germany).

Results: In *L. monocytogenes* strains isolated from food, resistance genes to cadmium (28,9%), arsenic (24,2%) and benzalkonium chloride (4,7%) were detected. The *cadA2C2* genes were found together with the *bcrABC* cassette in two strains, namely 3855-D (IIc, ST9, CC9) and 4315 (IVb, ST6, CC6). Furthermore, the *cadA4C4* genes, along with the *arsR1D2R2A2B1B2* genes encoding the arsenic cassette, were detected in 24.4% of the strains. The prevalence of cadmium resistance genes was comparable in strains from lineage I (IVb, IIb) and lineage II (IIa, IIc), namely, 85% and 15%. The prevalence of arsenic cassette was found in 9 strains of clonal complex CC2 (82%), and 1 strain CC 3 (9%) and 1 strain CC11 (9%).

Conclusions: The results of the present study demonstrate the need for further research into the characteristics of *L. monocytogenes* isolated from other sources in order to understand their spread throughout the food chain.

Disclosure: No significant relationships.

Keywords: L. monocytogenes,, heavy metal resistance,, genetic determinants, wgs

SHIFT 02-093

Topic: ***AS15. Food microbiology***

SOPPRESSATA OF MARTINA FRANCA STARTED WITH SELECTED BACTERIA AND FUNGI

Lecture Title:

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Background and Aims: Soppressata of Martina Franca is a dry-cured sausage, listed among the Italian agrifood products of cultural and gastronomic heritage. Despite a consolidated artisanal production process, the industrial production, requires improved safety and quality standards, reducing the use of chemical additives. The goal was to develop a standardized manufacturing process, mediated by autochthonous and/or selected microbial starters (bacteria and fungi).

Methods: This product was manufactured following the typical procedures adding selected bacteria and fungi. Microorganisms were previously screened in vitro and during seasoning trials for reducing nitrite content, proteolysis and lipolysis activities and counteracting food-borne pathogens, spoilage agents and mycotoxins producers. The genome and transcriptome of the screened autochthonous fungal species was sequenced and studied to characterize, secreted enzymatic activities and secondary metabolites production.

Results: *Staphylocoocus xylosus* ITEM18282 and *S. carnousus* ITEM18283 were selected for high nitrite reducing activity in the soppressata. Sausages treated with both selected staphylococci strains showed very low nitrite levels and significant increase of redness and tenderness. Among fungi, the autochthonous *Penicillium nalgiovense* ITEM18323, ensured homogeneous mycelial coverage, avoiding contamination by other spoilage or mycotoxigenic fungi, enhancing typical flavor and taste. The secreted pattern of proteins, enzymes and secondary metabolites predicted by *P. nalgiovense* ITEM18323 genome analysis, supports its beneficial use. Technological parameters (pH, Aw, ORP, weight loss), were consistent with the values of traditional soppressata of Martina Franca or other Italian long-seasoned dry-cured sausages.

Conclusions: This biotechnological protocol will allow to drive the production process of this soppressata, maintaining its traditional quality, ensuring safety even for industrial manufacturing.

Disclosure: No significant relationships.

Keywords: Fermented sausage, microbial starter, nitrite

SHIFT 02-094

Topic: ***AS15. Food microbiology***

IN VITRO CONTROL OF GNOMONIOPSIS SMITHOGILVYI: EVALUATION OF COMMERCIAL CHEMICAL AND BIOLOGICAL AGENTS

Lecture Title:

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Background and Aims: *Gnomoniopsis smithogilvyi* poses a significant threat to sweet chestnut (*Castanea sativa*) producers, being the primary cause of chestnut loss worldwide.The main objective of this study was to assess the in vitro effectiveness of various commercial fungicides in controlling *G. smithogilvyi* growth

Methods: Ten commercial products were tested with the active ingredient copper oxychloride, cuprous oxide, pyriofenone, phosphorus pentoxide, fenhexamide, potassium phosphonate, tebuconazole, *Bacillus mojavensis, Clonostachys rosea (*formerly *Gliocladium catenulatum* strain J1446) and *Gliocladium* G46 WG. Confrontation and plate diffusion tests were performed using four concentrations of commercial products to evaluate their efficacy against *G. smithogilvyi*. The growth of *G. smithogilvyi* was also evaluated in Potato Dextrose Agar (PDA) with the chemical agent incorporated into the medium.

Results: Tebuconazol, potassium phosphonate, *Gliocladium* G46 and *Bacillus mojavensis* were the most effective in controlling *G. smithogilvyi*. Additionally, products with copper oxychloride, cuprous oxide, and potassium phosphonate demonstrated a preventive effect against *G. smithogilvyi*.

Conclusions: In conclusion, tebuconazole and potassium phosphonate were the most effective against *G. smithogilvyi*, however, with the foreseen withdrawal from the European market, biological control agents such as *Gliocladium* G46, or similar, could be an alternative. AG and RM are grateful, respectively, to research (BI/UTAD/61/2023, under the cooperation protocol UTAD/Commercial Química Massó, S.A.) and PhD (BI/UTAD/80/2022) grants. The authors are thankful to FCT, Portuguese Foundation for Science and Technology, under the projects CITAB (UIDB/04033/2020), Inov4Agro (LA/P/0126/2020), CIMO (UIDB/00690/2020; UIDP/00690/2020) and SusTEC (LA/P/0007/2020).

Disclosure: The research was partially financed by a protocol with "Empresa Commercial Química Massó, S.A.", which made biological fungicides available to test their effectiveness against Gnomoniopsis smithogilvyi. The remaining products tested were purchased by us.

Keywords: Brown Rot, Chestnut orchard, Fungicides

SHIFT 02-095

Topic: ***AS15. Food microbiology***

EXPLORING THE MICROBIAL SECRETS: TECHNOLOGICAL AND PROBIOTIC INSIGHTS OF PORTUGUESE PDO CHEESES"

Lecture Title:

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Background and Aims: Portugal produces a diverse array of traditional raw milk cheeses, each with unique organoleptic characteristics. These products not only play a vital role in the local economy and cultural heritage but also harbor a rich microbiota, with lactic acid bacteria (LAB) being particularly prominent. LAB contribute to the safety of the final product and offer potential probiotic benefits. This study aims to assess the probiotic and technological attributes of LAB isolated from Portuguese raw milk cheeses.

Methods: LAB collected over several years were screened for hemolytic activity, while technological potential was examined through gelatinolytic, proteolytic, and lipolytic activities, as well as growth under different salt and temperature conditions. Probiotic potential was evaluated through viability assays at various pH levels, growth in the presence of bile, and assessment of activity against selected pathogenic indicator bacteria.

Results: Of the 42 selected isolates, 24% showed proteolytic activity, 55% displayed lipolytic ability, and 2% exhibited gelatinolytic features. The majority was capable of growing at high salt concentrations and across a range of temperatures, although fewer grew at refrigeration temperatures. Regarding probiotic potential, many isolates demonstrated the ability to inhibit pathogens and exhibited growth in bile-supplemented media. Viability was higher at higher pH levels, but remained promising even at pH 2.

Conclusions: These findings underscore LAB importance in cheese production and highlight their potential to enhance consumer health through their probiotic properties. **Acknowledgements:** FCT (Fundação para a Ciência e Tecnologia) under projects UIDP/00006/2020, LA/P/0059/2020 and PTDC/OCE-ETA/1785/2020 [EMOTION]. SSerrano holds a PhD-fellowship (FCT - UI/BD/153073/2022). MTBC: iNOVA4Health (UIDB/04462/2020; UIDP/04462/2020)

Disclosure: No significant relationships.

Keywords: Cheese microbiota, lactic acid bacteria, Technological potential, Cultural heritage, Probiotic potential

SHIFT 02-096

Topic: ***AS15. Food microbiology***

DEVELOPMENT OF IMPROVED METHOD TO ENHANCE THE SELECTIVE GROWTH, ISOLATION, AND IDENTIFICATION OF LISTERIA MONOCYTOGENES FROM FOOD CONTAINING HIGH MICROFLORA.

Lecture Title:

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Background and Aims: Foodborne Listeriosis is one of the most serious and severe foodborne illnesses caused by *Listeria monocytogenes*, especially from the consumption of uncooked food, such as microgreens and sprouts. These foods contain high microflora, which competitively diminishes the growth of *L. monocytogenes* and leads to false-negative results. The first aim of this study was to develop a modified Listeria Enrichment Broth (m-LEB) that selectively enhance the growth of *L. monocytogenes* in the presence of competing microbial flora and the second aim of the study was to establish a targeted sequencing procedure using the MinION sequencing platform to quickly detect *L. monocytogenes* in complex foods.

Methods: Fifty samples spiked with *L. monocytogenes* were grown in BHI overnight and DNA was extracted. Oxford nanopore sequencing was performed to determine the microbial flora composition using the RefSeq Masher and kraken2. The antimicrobial resistance (AMR) genes were identified using ResFinder and RGI. A modified LEB containing fosfomycin was formulated, and *Listeria* were identified using a targeted/adaptive metagenomic sequencing approach.

Results: All *Listeria* genomes contained *fosX* resistance genes that confer resistance to fosfomycin, whereas all normal flora samples lack it. In 28% of cases, m-LEB recovered *L. monocytogenes* better than regular LEB, proving that the presence of fosfomycin in the media provides a selective advantage for the recovery of Listeria. Targeted/adaptive sequencing showed 100% specificity and high sensitivity for the identification of *L. monocytogenes* against other background flora.

Conclusions: Modified LEB provides a selective growth advantage, and targeted sequencing provides an excellent tool for the specific identification of *Listeria monocytogenes*.

Disclosure: No significant relationships.

Keywords: AMR, Nanopore sequencing, Listeria, Fosfomycin, Modified LEB

SHIFT 02-097

Topic: ***AS15. Food microbiology***

IMPACT OF NITRITE REDUCTION ON THE MICROBIOLOGICAL SAFETY OF COOKED PORK HAM

Lecture Title:

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Background and Aims: Nitrite is a preservative used worldwide, particularly in meat products, to ensure quality and safety of food by inhibiting growth of pathogenic bacteria and protecting from spoilage. Cooked pork ham contains nitrite, which raises concerns regarding potential risks for the consumer's health, leading to changes in European legislation, reducing the limit allowed in pork ham from 150 mg/kg to 80 mg/kg of meat.

Methods: This study focuses on the assessment of the impact of nitrite reduction on the microbiological safety and organoleptic properties of pork ham. The determination of total viable counts (TVC), lactic acid bacteria (LAB), *Enterobacteriaceae, Escherichia coli* and pathogenic bacteria (*Listeria monocytogenes, Salmonella* and spores of sulphite-reducing *Clostridium*) was performed according to ISO standards in pork ham produced with different nitrite concentrations, during its shelf-life. Organoleptic characteristics, such as colour, a_w, pH and texture were evaluated at 0, 45 and 90 days.

Results: Although nitrite concentration decreased to almost half of its value, the microbiological results show that after 90 days of storage at 4 ºC, the product remained stable in compliance with the legal limits, below 1.0E+04 CFU/g for TVC and LAB, and absence of pathogenic bacteria.

Conclusions: In conclusion, although further tests are needed to establish safety, no significant differences were observed in microbial growth nor in organoleptic properties, during the product's shelf-life. Therefore, there is no evidence of safety problems associated with consumption of pork ham with a decrease higher than 45% in the nitrite concentration. However, caution should be taken until further research confirms its safety.

Disclosure: No significant relationships.

Keywords: Food safety, microbiology, nitrite, cooked pork ham

SHIFT 02-098

Topic: ***AS16. Food Mycology/Taxonomy Penicillium and Aspergillus***

DETERMINATION OF ROQUEFORTINE C, MYCOPHENOLIC ACID, AND VOLATILE COMPONENTS OF PENICILLIUM ROQUEFORTI ISOLATES FROM TURKISH BLUE CHEESES

Lecture Title:

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Background and Aims: Konya Kuflu and Erzurum Kuflu Civil are the most consumed mold-ripened cheeses in Turkiye, similar to European blue cheeses such as Roquefort, Stilton, Gorgonzola, and Danablu. These cheeses owe their distinctive taste, aroma, and blue-green color to the principal mold, *Penicillium roqueforti*. Mold-ripening in Turkish blue cheeses is a spontaneous process and does not involve starter cultures distinct from European counterparts. We aimed to determine the roquefortine C (ROQC) and mycophenolic acid (MPA), and the volatile components of *P. roqueforti* isolates obtained from Turkish blue cheeses.

Methods: In previous studies, the population genetics and phylogenetic analyses were conducted to determine the diversity of *P. roqueforti* isolates (n=120) isolated from 61 Turkish blue cheeses; 20 isolates was selected, and the technological properties of the isolates were examined. In this study, we will determine the volatile components and mycotoxin production of the 20 *P. roqueforti* isolates. Volatile profiles of the isolates will be analyzed using GC-MS. Mycotoxin analysis will be performed using HPLC. The target mycotoxins, ROQC and MPA, will be quantitatively detected.

Results: The volatile components and the production of ROQC and MPA of the 20 *P. roqueforti* isolates obtained from Turkish blue cheeses will be determined.

Conclusions: The volatile components and the production of ROQC and MPA of the *P. roqueforti* population in Turkish blue cheese will be examined. In further studies, model cheese production will focus on using the isolates selected based on metabolite analyses. *P. roqueforti* isolates from the Turkish blue cheese population could be used as secondary starters in cheese production.

Disclosure: No significant relationships.

Keywords: Turkish blue cheese, Penicillium roqueforti, roquefortine C, mycophenolic acid, volatile components

SHIFT 02-099

Topic: ***AS17. Gene expression, gene regulation and development***

LOSS OF HISTONE H3K27 METHYLATION ACCELERATES CELLULAR SENESCENCE IN TELOMERASE-DEFICIENT CELLS

Lecture Title:

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Background and Aims: Little is understood about how subtelomeric histone post-translational modifications are linked to telomere maintenance and integrity. We explored the genetic interactions between telomerase and histone modifiers in the budding yeast *Cryptococcus neoformans*.

Methods: *EST2* encodes a telomerase reverse transcriptase in the ascomycete yeast *Saccharomyces cerevisiae*. To confirm the role of an *EST2* ortholog in the basidiomycete yeast *C. neoformans*, we deleted its coding sequence using CRISPR-Cas9 genome editing and examined the viability of *est2Δ*. We then investigated the genetic interactions between telomerase activity and subtelomeric histone marks, H3K9me2/3, H3K27me3, and H4K20me3, by deleting *EST2* and the gene encoding the corresponding histone methyltransferase, *CLR4*, *EZH2*, and *SET9*.

Results: We observed that *est2Δ* progressively undergoes senescence and starts showing a significant loss of viability after a week from *EST2* deletion due to telomere shortening. Deletion of each histone methyltransferase did not affect cell viability, and when combined with *EST2* deletion, *clr4Δ est2Δ* and *set9Δ est2Δ* did not show a noticeable phenotypic change. Surprisingly, *ezh2Δ est2Δ* displayed a rapid loss of viability, suggesting a role of subtelomeric H3K27me3 in telomere protection. The accelerated cell death observed in *ezh2Δ est2Δ* was reproduced when *EST2* was deleted in *H3K27A* mutant cells.

Conclusions: We found that perturbation of subtelomeric H3K27me3 leads to accelerated cellular senescence and cell death in telomerase-deficient cells. Our data suggest that the Polycomb-mediated heterochromatin domains marked by H3K27me3 may be more directly involved in telomere maintenance and integrity than previously appreciated.

Disclosure: No significant relationships.

Keywords: Polycomb, Telomere, H3K27me3, Telomerase, Senescence

SHIFT 02-100

Topic: ***AS17. Gene expression, gene regulation and development***

THE INCREASED EXPRESSION OF RESPONSE REGULATOR CRRA IS IMPORTANT FOR OF MULTIDRUG EFFLUX PUMP KEXD EXPRESSION IN KLEBSIELLA PNEUMONIAE

Lecture Title:

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Background and Aims: The RND-type multidrug efflux system of Gram-negative bacteria is important for protecting them from antimicrobial agents. However, some of the multidrug efflux pumps are silent or expressed only at low levels. Genomic mutations often cause over-expression of such genes, and bacteria acquire resistance to multiple antibiotics, biocides, and harmful chemicals at once. We previously reported mutants with increased expression of the multidrug efflux pump gene *kexD*, one of the silent genes.

Methods: Transposon (Tn) mutagenesis was performed to investigate the cause of the overexpression of KexD. Comparisons between the results of qPCR were tested using Dunnett's test or t-test.

Results: A mutation in CrrB was identified in KexD-overexpressing mutants. CrrB is presumed to be a histidine kinase of a two-component regulatory system.
The expression of CrrA, a response regulator increased in the mutant overexpressing KexD, and the strains in which CrrA was complemented by a plasmid showed elevated expression of KexD and CrrB from the genome. The complementation of the mutant-type CrrB also increased the expression of KexD and CrrA from the genome, but the complementation of the wild-type CrrB did not. Therefore, the expression levels of CrrA may directly affect the increased expression of KexD.

Conclusions: Our results emphasized the importance of the CrrAB system in controlling the expression of KexD. Whereas, various levels of CrrA expression were observed in other Tn-inserted strains, possessing Tn in locations other than *crrB*, though their expression levels of KexD were decreased by Tn-insertion. This result suggests other factor(s) exist to affect the expression of KexD.

Disclosure: No significant relationships.

Keywords: RND-type multidrug efflux pump KexD, two-component regulatory system

SHIFT 02-101

Topic: ***AS18. Genomics and functional genomics***

SIMIULTANEOUS ASSIMILATORY AND DISSIMILATORY NITRATE REDUCTION IN BACILLI STRAINS ISOLATED FROM THE RICE PADDY FIELD SOIL IN SOUTH KOREA

Lecture Title:

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Background and Aims: Denitrification and dissimilatory nitrate reduction to ammonium (DNRA) were thought to be constrained to anoxic condtions. However, the soil bacilli strains isolated from the rice paddy field soil in this study were observed to reduce NO_3^- and produce extracellular NH_4^+ regardless of oxygen presence. The study is for figuring out that these soil bacterial strains actually perform denitrification and DNRA under aerobic conditions.

Methods: The soil strains were cultured in a complex and defined minimal medium supplemented with NO_3^- and acetate. The colorimetric plate screening method was used to measure NO_3^-, NO_2^-, and NH_4^+ from the culture supernatant. Total RNA was extracted from cell cultures and reversly transcibred into cDNA for transcription analysis. Based on whole genomes seqeunces of the strains, the oligonucleotide primer sets were designed for functional genes and used for transcript analysis.

Results: The strains possess denitrification and DNRA functional genes on their genomes, and cotranscribed the genes such as *nirK*, *nosZ*, *nirB*, and *nrfA* simultaneously under aerobic condition. All results highlighted that the three facultative anaerobic strains reduce NO_3^- in assimilatory and dissmilatory pathways under both aerobic and unaerobic conditions.

Conclusions: *Neobacillus* spp. PS2-9 and PS3-12, and *Bacillus salipaludis* PS3-36 are soil bacterial strains performing assmilatory and dissmilatory NO_3^- reduction under aerobic and anerobic conditions. This is a novel report describing coexistence of denitritification, DNRA, and assmiliatory NO_3^- reduction in soil bacilli strains, which are valuable mibrobial resources to be studied for regulation of their nitrogen metabolism and roles played in soil nitrogen cycle.

Disclosure: No significant relationships.

Keywords: nitrate assimilation, denitrification, DNRA, soil bacilli, cotranscription

SHIFT 02-102

Topic: ***AS18. Genomics and functional genomics***

PHENOTYPIC ANALYSIS OF GGDEF/EAL DOMAIN PROTEIN FUNCTIONS IN PHYTOPATHOGENIC PANTOEA ANANATIS

Lecture Title:

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Background and Aims: Cyclic diguanosine monophosphate (c-di-GMP) is a widely distributed bacterial signaling molecule that regulates various cellular processes such as colony morphology, motility, biofilm formation, and virulence. C-di-GMP is synthesized by diguanylate cyclases (DGCs) with the GGDEF domain and degraded by phosphodiesterases (PDEs) with the EAL domain. Here, we identified 26 predicted c-di-GMP metabolism-related genes in the genome of a plant pathogenic bacterium *Pantoea ananatis* PA13: nine genes encoding GGDEF-only domain, five encoding dual GGDEF/EAL domains and twelve encoding EAL-only domain.

Methods: We constructed overexpression and mutant strains of all DGC- or PDE-encoding genes and assessed their Congo Red (CR) binding, mucoid and rugose phenotypes, pellicle formation, and swimming motility.

Results: We identified 14 out of 26 DGC or PDE proteins affecting phenotype changes. Of the 26 DGC- or PDE-overexpressing strains, 8 strains increased CR-binding and induced pellicle formation; 5 strains reduced mucoid formation, and 2 strains increased it; 5 strains caused rugose colony morphotype alteration; and 7 strains decreased swimming motilities. Only the DgcM-overexpression strain displayed bacterial sessile behaviors, including reduced mucoid and motility, induction of pellicle formation and rugose colony morphology, and enhanced CR-binding. Among all DGC- or PDE-encoding gene mutants, the *pdeC* mutant showed induction of cellulase-sensitive pellicle formation and increased CR-binding, the *pdeM* mutant reduced mucoid phenotype, and the *pdeS* mutant slightly increased swimming motility. DGC-overexpression and PDE-encoding gene mutant strains exhibiting phenotypic changes produced higher c-di-GMP levels than the wild type.

Conclusions: This study provides essential information for understanding the role of the c-di-GMP network in *P. ananatis*.

Disclosure: No significant relationships.

Keywords: Pantoea ananatis, c-di-GMP, phosphodiesterase, diguanylate cyclase, pellicle

SHIFT 02-103

Topic: ***AS18. Genomics and functional genomics***

STUDY ON THE EFFECT OF MUTANT HC-PRO ON THE PATHOGENICITY OF A PROTECTIVE MILD STRAIN OF PEPPER VEINAL MOTTLE VIRUS

Lecture Title:

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Background and Aims: HC-Pro is a pathogenicity determinant of potyviruses. A mild zucchini yellow mosaic virus (ZYMV) strain ZAC obtained from wild-type (WT) ZYMV through point mutations caused mild and recovery symptoms on squash plants and no local lesions on quinoa leaves. ZAC was engineered as an expression vector to study the pathogenicity-related activity of heterologous viral proteins. Another attenuated pepper veinal mottle virus (PVMV) strain m4-8 was obtained through nitrite-induced mutagenesis. An 18-aa residue difference between the genomes of m4-8 and its WT virus was found. Two aa residue mutations, $K_{117}E$ and $K_{282}R$, were identified within HC-Pro. In this study, m4-8 HC-Pro was expressed by ZAC to study its pathogenicity-related activity.

Methods: The HC-Pro ORF of WT PVMV and its mutant m4-8 were amplified by RT-PCR and inserted into ZAC vector to generate recombinant clones pZAC-Tn-HC and pZAC-m4-8-HC that were subsequently introduced into squash and leaves. The recovered viruses were named ZAC-Tn-HC and ZAC-m4-8-HC, respectively. WT ZYMV and ZAC-GFP, an attenuated ZAC recombinant carrying GFP ORF, were also inoculated onto plants for comparison. The recombinant viruses were verified through RT-PCR and amplicon sequencing.

Results: On leaves, ZAC-m4-8-HC, similar to ZAC-GFP, caused no local lesions. In contrast, ZAC-Tn-HC induced chlorotic lesions, but differently from those caused by WT ZYMV. ZAC-Tn-HC and ZAC-m4-8-HC caused similar mottle symptoms on squash plants but were different from those caused by ZAC-GFP and WT ZYMV.

Conclusions: The aa residues K_{117} and K_{282} of HC-Pro may be involved in, but not essential for, the pathogenicity of PVMV.

Disclosure: No significant relationships.

Keywords: Potyvirus, HC-Pro, pathogenicity

SHIFT 02-104

Topic: ***AS18. Genomics and functional genomics***

STUDY ON PATHOGENIC DETERMINANTS OF TOMATO YELLOW LEAF CURL THAILAND VIRUS

Lecture Title:

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Background and Aims: Tomato yellow leaf curl disease (TYLCD) causes severe tomato losses worldwide. Tomato yellow leaf curl Thailand virus (TYLCTHV), a bipartite *Begomovirus* species transmitted by whiteflies, is the main causal agent of TYLCD in Taiwan. Many previous studies showed that AC2, AC4 and AV2 function as gene-silencing suppressors and pathogenicity determinants of bipartite begomoviruses.[MO使1] In this study, an attenuated vector ZAC constructed from infectious clone of zucchini yellow mosaic virus (ZYMV) with point mutations within HC-Pro, a suppressor of RNA-silencing and pathogenicity determinant, was used to investigate the pathogenicity-related activity of AC2, AC4, and AV2 of TYLCTHV.

Methods: The AC2, AC4 and AV2 ORFs of TYLCTHV were amplified by PCR and inserted into ZAC vector to generate recombinant clones. The clones were mechanically introduced into squash and quinoa leaves. Symptoms caused by the recovered viruses, named ZAC-THV-AC2, ZAC-THV-AC4 and ZAC-THV-AV2, respectively, were observed. Wild-type (WT) ZYMV and its attenuated recombinant ZAC-GFP carrying GFP ORF were also inoculated onto squash and quinoa plants for comparison. The recombinant viruses were verified through RT-PCR and amplicon sequencing.

Results: Unlike WT ZYMV-induced chlorotic lesions, ZAC-THV-AC2, ZAC-THV-AC4 and ZAC-THV-AV2, similar to ZAC-GFP, caused no local lesions on quinoa leaves. ZAC-THV-AC2 and ZAC-THV-AC4 induced similar responses to ZAC-GFP in squash plants. In contrast, ZAC-THV-AV2 caused mosaic symptoms but were apparently different from those caused by WT ZYMV.

Conclusions: ZAC-THV-AV2 partially restored the virulence of ZAC in squash, suggesting that the TYLCTHV AV2 protein may act as a pathogenicity determinant.

Disclosure: No significant relationships.

Keywords: Begomovirus, pathogenicity, ZYMV vector

SHIFT 02-105

Topic: ***AS18. Genomics and functional genomics***

GENOMIC ANALYSIS OF PENICILLIUM CRUSTOSUM ISOLATES CONTAMINATING NATURAL BLACK TABLE OLIVES.

Lecture Title:

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Background and Aims: Table olives are a fermented food widely consumed in Mediterranean countries. Molds growth on the brine surface should be carefully considered as potential spoilage and mycotoxins contaminants. Here we investigate the genome of two *Penicillium crustosum*, isolated from Italian and Greek fermented black table olives, to characterize the enzymatic activities and metabolites, that can affect quality and safety traits of the final product.

Methods: Sequencing and annotation of the genome of *P. crustosum* ITEM16721 and ITEM16753 was done by collaboration involving JGI, PNNL and CNR-ISPA. Draft genomes and transcriptomes were generated using Illumina technology. Nuclear genomes were annotated using the JGI Annotation pipeline. Protein-coding gene models were generated and functionally annotated using a combination of *ab initio*, homology-based, and transcriptome-based gene predictors. Protein domains, transcription factors and secondary metabolites, have been catalogued. Gene clusters, and cellular localization of proteins with interesting catalytic activities were also predicted. Furthermore, the potential for mycotoxins production was also evaluated.

Results: The draft genome assembly yielded in 112 to 144 scaffolds and 11506 to 11388 protein-coding genes for *P. crustosum* ITEM16721 and ITEM16753, respectively. Protease, lipase, esterase, and carbohydrate-active enzymes have been catalogued along with other interesting catalytic activities. Mycotoxin and biosynthetic clusters were identified.

Conclusions: The availability of the genomic assembly and annotation will facilitate advancement in knowledge of *P. crustosum* biology, both from an evolutionary perspective and in terms of its role in fermentation processes. Genomic features of *P. crustosum* confirm safety and quality issues of table olives.

Disclosure: No significant relationships.

Keywords: Secondary metabolites, enzymes, Genomics, Transcriptomics, Fermented olives

SHIFT 02-106

Topic: ***AS18. Genomics and functional genomics***

PHYLOGENOMICS AND ANALYSIS OF ANTIMICROBIAL RESISTANCE GENES OF 72 ACINETOBACTER SPECIES

Lecture Title:

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Background and Aims: Non-*baumannii Acinetobacter* species are increasingly isolated in the clinical setting and the environment. The aim of the present study was to analyze a database of 837 *Acinetobacter* spp. genomes, in order to define the concordance of classification and discriminatory power of Pasteur and ribosomal MLST, and single-nucleotide polymorphism (SNPs) phylogenies.

Methods: Bacterial genomes were manually selected from the PubMLST database. Phylogenies were performed on Pasteur or ribosomal MLST concatenated alleles, or SNPs extracted from core genome alignment using RAxML. The resistance genes were detected using ABRicate software.

Results: The Pasteur MLST scheme was able to identify and genotype 72 species in the *Acinetobacter* genus, with classification results concordant with the ribosomal MLST scheme. The discriminatory power and the genotyping reliability of Pasteur MLST scheme was assessed in comparison to genome-wide SNP phylogeny on 535 non-*baumannii Acinetobacter* genomes assigned to *A.pittii*, *A.nosocomialis*, *A.seifertii* and *A.lactucae*, which are the most clinically relevant non-*baumannii* species of the *A. baumannii* group. The Pasteur MLST and SNP phylogenies were congruent and grouped genomes into four monophyletic and six non-monophyletic clades in *A. pittii*, and one each in *A. seifertii*, respectively. Also, *A. lactucae* genomes were grouped into one non-monophyletic clade within *A. pittii* genomes. The SNP phylogeny of *A. nosocomialis* genomes showed a heterogeneous population and did not correspond to Pasteur MLST phylogeny. Acquired antimicrobial resistance genes analysis classified MultiDrugResistant 35 *Acinetobacter* species.

Conclusions: The Pasteur MLST scheme is a useful genotyping tool to identify *Acinetobacter* species and to analyze the population structure of the *A. baumannii* group.

Disclosure: No significant relationships.

Keywords: Acinetobacter spp., Pasteur Multilocus Sequence Typing, Antimicrobial resistance genes, Maximum-likelihood phylogeny, Ribosomal Multilocus Sequence Typing

SHIFT 02-107

Topic: ***AS19. Host-pathogen interactions***

IN SITU CYTOKINE EXPRESSION AND SOLUBLE C-TYPE LECTIN RECEPTORS LANDSCAPE IN RECURRENT VULVOVAGINAL CANDIDIASIS

Lecture Title:

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Background and Aims: We previously reported that patients suffering recurrent vulvovaginal candidiasis (RVVC) showed diminished fungicidal and proliferative capacities of neutrophils and peripheral blood mononuclear cells, respectively, suggesting that the innate and adaptive immune responses are altered. We aimed to determine the expression of genes associated with the immune response and the soluble C-type lectin receptors in patients with RVVC.

Methods: A cross-sectional study was carried out on 40 patients with diagnosis of RVVC and 26 healthy women. To evaluate gene expression by qPCR, vaginal scrapings were obtained from patients and controls, respectively. Genes coding for cytokines, transcription factors specific for Th1, Th2, Th17 and Treg profiles, and for enzymes related to oxidative/microbicidal mechanisms were evaluated. Additionally, dectin-1 and mannose binding lectin (MBL) soluble receptors were also determined in serum from patients and controls by ELISA.

Results: We observed that patients with RVVC showed a decreased expression of T-bet, ROR-γT, IL-1β, and IL-17, while a higher expression of FoxP3, IL-4, IL-8, IL-10, and IL-18 was noted in those patients compared to healthy women. Additionally, RVVC patients exhibited a lower but significant level of MBL compared to healthy women.

Conclusions: Women affected by RVVC showed increased expression of both pro- and anti-inflammatory cytokines; of note, RVVC patients showed a decrease in the expression of hallmark cytokines and transcription factors relating to Th1 and Th17 profiles; moreover, diminished levels of soluble MBL were also observed in those patients. Altogether, these data confirm an alteration in the innate and adaptative immune response in patients suffering RVVC.

Disclosure: No significant relationships.

Keywords: Recurrent Vulvovaginal Candidiasis, Immune response, Cytokines, C-type lectins

SHIFT 02-108

Topic: ***AS19. Host-pathogen interactions***

BLOOD MICROBIOTA SCREENING FOR PATHOGENS IN PREVALENT BAT SPECIES IN NORTHERN BULGARIAN CAVES

Lecture Title:

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Background and Aims: Out of 47 European bat species 33 inhabited Bulgaria. Recently, bats have been identified as an important reservoir for numerous emerging zoonotic viruses such as SARS, Ebola, Nipah, Hendra, Marburg, and MERS. Individual bacterial, fungal, and protozoa pathogens have been described. However, there is no data about the blood microbiome of bats and its capacity to generate zoonotic spillover, which was the aim of our study.

Methods: We analysed blood samples of bat species of *Vespertilionidae* and *Rhinolophidae* families inhabiting three caves in northern Bulgaria with 80 – 130 distance from each other. 30 µl of blood was collected from each animal. The blood of 120 bats was pooled in 13 samples. DNA was extracted. Illumina shotgun and 16S/18S/ITS full-length amplicon-based metagenomics were performed. Taxonomic identification of the cleaned raw reads was performed by Kraken2 with Refseq NCBI database for Prokaryote, Protozoa Fungi and viruses on www.usegalaxy.eu. OTU table was generated with the Bracken.

Results: A significant number of microbial pathogenic species were identified in the bat's blood. Sequencing reads exceeding 10K were identified for Mycobacteria, Bartonella, Plasmodium, and Leishmania species. Other pathogenic species such as Toxoplasma spp. have also been identified but less evidently according to the number of reads. While tuberculosis, bartonellosis, and leishmania are rare diseases, malaria is almost absent in Bulgaria. Moreover, the bat's blood microbiota diversity was dependent stronger on the host taxonomy than the geographic location.

Conclusions: Our results demonstrated that bats in Bulgaria are a hidden or potential reservoir for malaria and toxoplasmosis. Acknowledgments: Grant KP-06-PN-51/9-2021

Disclosure: No significant relationships.

Keyword: bat, blood microbiome, bartnelosis, zoonoses, metagenomics

SHIFT 02-109

Topic: ***AS19. Host-pathogen interactions***

THE CELL TYPE-SPECIFIC SUSCEPTIBILITY AND GENETIC ANALYSIS OF MONKEYPOX VIRUS CLINICAL ISOLATES MPXV-ROK-P1-2022.

Lecture Title:

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Background and Aims: Monkeypox virus (MPXV) is a member of the *Orthopoxvirus* genus belonging to the family *Poxviridae*, which includes variola virus, the causative agent of smallpox. Following the global outbreak of MPXV declared by the World Health Organization (WHO) in 2022, MPXV-ROK-P1-2022 was isolated and cultured from the first reported patient in South Korea by the Korean Centers for Disease Control and Prevention, and it has been identified as belonging to the West African clade IIb B.1.1. lineage. Here, we assessed the viral susceptibility, cytopathic effects, and genetic analysis of MPXV-ROK-P1-2022 in various cell lines to understand their characteristics.

Methods: MPXV's wild type and inactivated virus are inoculated into various human and animal host cell lines. Viral susceptibility and cytopathic effects are observed, and through analyses of virus replication and gene expression, the infection characteristics of the virus are elucidated based on cell type.

Results: MPXV-ROK-P1-2022 showed robust growth in the Vero E6 cell line, in which it was originally isolated and cultured, as well as significantly higher levels of viral titer in other cell lines such as MRC-5, which had been reported with other clinical isolates. By analyzing viral susceptibility and transcriptome across various cell lines, we were able to elucidate the infection characteristics and underlying mechanisms of action of MPXV-ROK-P1-2022 in different hosts.

Conclusions: Through this study, we were able to confirm the characteristics of the MPXV-ROK-P1-2022 clinical isolates and acquire key genetic information involved in virus infection and host immune responses.

Disclosure: No significant relationships.

Keywords: monkeypox virus, Susceptibility

SHIFT 02-110

Topic: ***AS19. Host-pathogen interactions***

TRIPARTITE MOTIF-CONTAINING PROTEIN 21 MEDIATES THE INTERFERON-GAMMA INDUCED SUPPRESSION OF HEPATITIS B VIRUS

Lecture Title:

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Background and Aims: The antiviral role of the tripartite motif (TRIM) family protein, a member of the E3-ubiquitin ligase family, has recently been actively studied. Hepatitis B virus (HBV) infection is a major contributor to liver diseases; however, the host factors regulated by cytokine-inducible TRIM21 to suppress HBV remain unclear.

Methods: We tested the ability of TRIM21 to fight HBV in liver cell lines, primary human liver cells, and mice. Knock-out cells helped confirm the impact of TRIM21 on HBV suppression, especially when IFN-γ is involved. We checked HBV RNA levels via Northern blot and assessed HBV enhancer activity with Luciferase reporter assays. Deletion mutant experiments clarified how different parts of TRIM21 contribute to its anti-HBV effects. Protein-protein interactions were identified through co-immunoprecipitation, while the role of TRIM21 in protein degradation was evaluated via ubiquitination assays.

Results: Our findings confirmed that TRIM21 diminishes HBV replication by reducing HBV RNA levels and suppressing the activity of HBV enhancers. Deletion mutant experiments revealed the importance of the RING domain and PRY-SPRY domain in the anti-HBV effect of TRIM21. Furthermore, we identified a novel interaction between TRIM21 and HNF4α, with TRIM21 promoting the ubiquitin-mediated proteasomal degradation of HNF4α.

Conclusions: The reduction of key HBV enhancer activators, HNF4α and HNF1α, by TRIM21 resulted in a decline in HBV transcription, ultimately leading to the inhibition of HBV replication. These findings highlight the importance of TRIM21 in the host's defense against HBV infection and provide insights into potential therapeutic strategies targeting TRIM21 for the treatment of HBV-related liver diseases.

Disclosure: No significant relationships.

Keyword: Hepatitis B virus, Tripartite motif-containing protein 21, Interferon-gamma

SHIFT 02-111

Topic: ***AS19. Host-pathogen interactions***

INVOLVEMENT OF LIPIDS IN NOROVIRUS REPLICATION IN HUMAN INTESTINAL ORGANOIDS

Lecture Title:

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Background and Aims: Human norovirus (HuNoV) frequently causes large outbreaks of foodborne disease worldwide. Some reports insisted that intestinal metabolism and absorption impacted the HuNoV infection. Recently, we found that bile was required for infection of some HuNoV genotypes in human intestinal organoids (HIOs) in a genotype-dependent manner (Murakami et al, PNAS, 2020). We also found that a specific lipid (lipid X) enhanced the HuNoV infection at the viral RNA levels (unpublished data). However, it is unclear whether the lipid affects the expression of viral proteins and the life cycle of HuNoVs. Here, we conducted some experiments to evaluate the role of the lipid in HuNoV infection in the intestine.

Methods: HIOs were infected with genogroup II, genotypes 3 and 4 (GII.3 and GII.4) HuNoVs in the presence of lipids of interest. The effect of lipids on HuNoV replication in the HIOs was analyzed by RT-qPCR, western blotting and immunofluorescence microscopy.

Results: The number of viral RNA copies was markedly increased in the presence of lipid X at 12 hours post-infection when the viral replication in the HIOs normally reached a plateau. The production of viral capsid proteins (VP1) was also increased by the addition of lipid X, judged from the results of western blotting and immunofluorescence microscopy. These results revealed that lipid X enhanced virus replication.

Conclusions: We raise the possibility that lipid X is required for enhanced replication of HuNoV in HIOs.

Disclosure: No significant relationships.

Keywords: replication, norovirus, lipid

SHIFT 02-112

Topic: ***AS19. Host-pathogen interactions***

INTRACELLULAR ACETYL-COA FLUX OCCURS THE KAPOSI'S SARCOMA

Lecture Title:

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Background and Aims: Kaposi's sarcoma (KS), which is induced by Kaposi's sarcoma-associated herpesvirus (KSHV), is a multicentric tumor of lymphatic endothelial origin that mainly involves the skin, lymph nodes, and visceral organs. However, the underlining molecular mechanisms of how the malignancy events leading to disseminated KS occur remain unclear.

Methods: Mass spectrometry analysis revealed that vIRF3 interacted with pyruvate kinase M2 (PKM2), which plays a pivotal role in glycolysis in converting phosphoenolpyruvate (PEP) to pyruvate, linking to ATP production and subsequent acetyl-CoA formation for the TCA cycle.

Results: Mechanistically, KSHV vIRF3 elevated intracellular acetyl-CoA levels, prompting acetylation of SMAD3, which contributes to the endothelial mesenchymal transition (EndMT) of LECs. We have also shown that recombinant KSHV-infected LEC can successfully induce tumorigenesis in a xenograft model, which notably represents the histopathological characteristics of disseminated KS. More interestingly, the disseminated organs in the xenograft were highly correlated with human KS patient statistics. Mechanistically, we also revealed that KSHV vIRF3 enhances cellular acetyl-CoA that promotes SMAD3 acetylation for the endMT of LEC.

Conclusions: Collectively, these results suggest that the crucial role of vIRF3 in KS malignancy causally links metabolic reprogramming of acetyl-CoA metabolism, EndMT, and cancer progression.

Disclosure: No significant relationships.

Keyword: KSHV, metabolic pathway, acetyl-CoA, EndMT

SHIFT 02-113

Topic: ***AS19. Host-pathogen interactions***

APPLICATION OF HUMIC SUBSTANCES DURING BIVALVE DEPURATION: EFFECTS ON THE GUT BACTERIOME OF CARPET SHELL CLAM (RUDITAPES DECUSSATUS).

Lecture Title:

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Background and Aims: Depuration stands as a vital process in ensuring the safety and quality of commercial clams for human consumption. Coadjutants for depuration are sought out to improve safety and shelf-life of seafood products and may include chemical agents with chelating and microbiome modulating capabilities. In this context, humic substances (HS) emerge as a cost-efficient and environmentally friendly solution to enhance depuration and modulate shellfish microbiome. In this study, we evaluated the potential of HS as a depuration coadjutant in modulating animal-host bacterial communities.

Methods: Two depuration systems, each with three replicate tanks, were executed during twenty-six hours. In one system, no coadjutant was added, while in the other, a water-soluble HS (sHsHumic Powder, FulviXcell) was added at final concentration of 2.5mgL^{-1}. After depuration, clams were stored in a climate chamber (5 ± 1 °C) for 6 days to simulate the commercial period ('shelf-life'). DNA was extracted from gut of clams sampled before depuration, after depuration and after 'shelf-life'. Bacterial composition was analysed using high-throughput sequencing data of the 16S gene.

Results: HS supplementation did not alter alpha-diversity, but both HS addition and storage were significant predictors of bacterial community composition. Bacterial communities were dominated by obligate intracellular symbionts, i.e, *Mycoplasma* sp. and *Ehrlichia* sp.. A Boruta feature-selection analysis detected family Metamycoplasmataceae as a significant predictor of experimental variables, whose abundance was highest in HS-depurated clams.

Conclusions: Overall, results reveal that the potential of HS in modulating clam microbiome, including intracellular host symbionts, should be further explored for both food science and ecosystem restoration.

Disclosure: No significant relationships.

Keywords: Humic Substances, Depuration, Microbiome Modulation, Food safety, Mycoplasma

SHIFT 02-114

Topic: ***AS19. Host-pathogen interactions***

ADVANCED IN VITRO MODELLING OF BACTERIAL PNEUMONIA: FROM LITERATURE TO LAB

Lecture Title:

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Background and Aims: Bacterial pneumonia greatly contributes to lower respiratory tract infection burden and mortality across demographics. Laboratory modelling of bacterial pneumonia remains important for elucidating host-pathogen interactions and assessing drug efficacy and toxicity. *In vitro* cell culture facilitates high-throughput disease modelling in controlled environments. Advanced human cell culture models, such as air-liquid interface (ALI), organoids, spheroids, and organ-on-chip models can bridge the research gap between classical cell models and animal models, contributing to innovative and comprehensive research methodology.

Methods: In the first step, we performed an extensive literature review of research articles (2003-2023) incorporating advanced cell culture models for bacterial pneumonia (Mahieu *et al*., 2024). We identified cell types, infection methods, and strategies for the validation of these models. Currently, our ongoing research is focused on the development of a bacterial-infected ALI model using our self-isolated collection of patient-derived primary lung epithelial cells and over 600 clinical *Streptococcus pneumoniae* isolates.

Results: Our literature review highlighted the need for validated and transparent protocols for the establishment of advanced bacterial lung infection models. Therefore, we first optimized and validated a protocol for the development of an advanced cell model. Using a well-defined antibody panel for basal, ciliated, secretory, and goblet cells to characterize variations in cell composition between different cell isolation methods (brushing vs. enzymatic digestion), immediately after isolation and 7-14 days post-expansion of the cells were investigated.

Conclusions: We will provide an extensive overview of advanced cell culture models and demonstrate their integration into our research efforts aimed at developing them to study bacterial pneumonia.

Disclosure: No significant relationships.

Keywords: Bacterial pneumonia, in vitro, advanced cell culture models, primary cells

SHIFT 02-115

Topic: ***AS19. Host-pathogen interactions***

VACUOLAR PROTEIN SORTING HOMOLOG B, RESPONSIBLE FOR COHEN SYNDROME, IS INVOLVED IN BARRIER FUNCTION AGAINST LIPOPOLYSACCHARIDE AND PEPTIDOGLYCAN IN HUMAN GINGIVAL EPITHELIAL TISSUES.

Lecture Title:

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Background and Aims: Gingival tissues provide the first line of defense against periodontal pathogens. We previously reported that coxsackievirus and adenovirus receptor (CXADR), a tight junction-associated protein, is involved in barrier function in gingival epithelial tissues, which prevents penetration of virulence factors such as lipopolysaccharide (LPS) and peptidoglycan (PGN). The mutation of *vacuolar protein sorting homolog B* (*VPS13B*) causes Cohen syndrome, an autosomal recessive disease, which develops various complications including periodontitis. However, the cause-and-effect relationship between *VPS13B* mutation and the etiology of periodontitis remains unclear. The aim of this study was to clarify the effects of VPS13B on gingival barrier function against periodontal pathogens.

Methods: We used immortalized human gingival epithelial cells wild type and Δ*VPS13B,* established by genome editing with the Crispr-Cas9 system. Three-dimensional tissue model was constructed by cell-accumulation technique. Intracellular localization and the protein levels were analyzed by confocal microscopy and immunoblotting.

Results: VPS13B was co-localized with CXADR. In Δ*VPS13B* cells, the level of CXADR protein was decreased, whereas mRNA level was not significantly changed when compared to the wild type ($p>0.1$). Additionally, mis-sorting of CXADR to lysosome was observed in Δ*VPS13B* cells treated with bafilomycin A1, an inhibitor against lysosomal degradation. The permeability to LPS and PGN in a Δ*VPS13B* tissue model was increased by 1.5-fold and 8.9-fold, respectively (n=8, $p<0.001$).

Conclusions: Our results suggest that VPS13B is involved in barrier function of gingival epithelial tissues via intracellular trafficking of CXADR. This study demonstrates the molecular basis to understand the etiology of periodontitis in patients with Cohen syndrome.

Disclosure: No significant relationships.

Keywords: VPS13B, Periodontal Disease, CXADR, Lipopolysaccharide, peptidoglycan

SHIFT 02-116

Topic: ***AS19. Host-pathogen interactions***

ESTIMATING PNEUMOCOCCAL SEROTYPE CARRIAGE DYNAMICS IN ADULTS WITH AND WITHOUT HIV DURING MATURE INFANT PNEUMOCOCCAL CONJUGATE VACCINE PROGRAMME IN MALAWI

Lecture Title:

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Background and Aims: People living with HIV (PLHIV) are at an increased risk of pneumococcal carriage and disease compared to the general population. However, the acquisition and clearance rates of pneumococcal carriage and their associated factors among PLHIV are poorly understood.

Methods: We collected weekly longitudinal nasopharyngeal swabs from HIV-uninfected, PLHIV ART<3-months and PLHIV ART>1-year adults. All participants were sex and age-matched and had an under-five in their household. Pneumococcal carriage was detected by microbiological culture. We fitted pneumococcal serotype carriage and covariates, including age, sex, pneumococcal density, and number of children, to multistate Markov models to capture the dynamics of pneumococcal carriage acquisition and clearance.

Results: 126 adults (2,007 follow-up samples) were equally enrolled into the three groups. The median age was 31(interquartile range:23-36), and 75% had one child. Baseline pneumococcal carriage was 31.0%, 26.2% and 50.0% for HIV-uninfected, PLHIV ART<3m and PLHIV ART>1year, respectively. Pneumococcal density was greatest among PLHIV ART<3m. Acquisition of 13-valent pneumococcal conjugate serotypes (VT) was higher in females (hazard ratio:2.45, 1.03-5.81) and households with >2 children (2.46,1.30-4.65). Non-VT clearance was slower among 35-44years than 18-34years (clearance ratio:0.18, 0.07-0.45) and among PLHIV ART>1year (0.29, 0.12-0.71). Serotype acquisitions were highest for VT 19F, 3 and 6A/B, and non-VT 23A/B, 15A/B/C/F and 11A/B/C/D/F, with types 6A/B, and 7A/B/C being carried longer.

Conclusions: PLHIV ART>1year have prolonged non-VT carriage duration, due to poor clearance, which drives the high carriage prevalence among these individuals. Our findings provide estimates for comparison after introducing optional vaccine strategies in PLHIV in high pneumococcal transmission settings, such as Malawi.

Disclosure: No significant relationships.

Keywords: Pneumococcal carriage duration, People Living with HIV, Multistate Markov Model, Pneumococcal Conjugate Vaccine

SHIFT 02-117

Topic: ***AS19. Host-pathogen interactions***

MOLECULAR ARMS-RACE BETWEEN MPXV VIDR AND RIPK3, A CENTRAL MEDIATOR OF NECROPTOSIS.

Lecture Title:

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Background and Aims: Monkeypox virus (MPXV, *Orthopoxvirus* genus) and other poxviruses encode proteins that inhibit necroptosis, an inflammatory form of cell death mediated by the receptor interacting protein kinase 3 (RIPK3) and its downstream effector MLKL. Orthopoxviruses counteract necroptosis at two levels: by blocking the ZBP1 factor (a trigger of necroptosis) via the E3 protein and by targeting RIPK3 to degradation through the vIRD (viral inducer of RIPK3 degradation) protein (J3L/J1R in MPXV). *RIPK3*$^{-/-}$ mice succumb to vaccinia virus infection, which is instead controlled in wt animals. Also, deletion of vIRD results in attenuation of cowpox virus.

Methods: The evolution of vIRD in orthopoxviruses and of RIPK3 in mammals was analyzed by codon-based codeml program (PAML suite) searching for positive selection signals. Positively selected sites were determined by using different methods (BEB, FEL and MEME). To study the more recent history of vIRD during MPXV dispersal in Africa, we applied gammaMap that jointly uses intra-specific variation and inter-specific diversity.

Results: We obtained solid evidence that both proteins were targets of positive selection. Most selected sites in vIRD are located in the N-terminal ankyrin-repeats domain (vIRD^{ank}), which is sufficient to bind RIPK3. Interestingly, one such site (188) is polymorphic in MPXV strains circulating in West and Central Africa and we determined that it evolved adaptively since the emergence of MPXV as a species. In RIPK3, many selected sites surround the RHIM motif, the region involved in vIDR binding.

Conclusions: This evolution-guided approach can provide insight into host-virus interactions, finding potential determinants of MPXV host range and virulence

Disclosure: No significant relationships.

Keywords: Monkeypox virus (MPXV), RIPK3, necroptosis, host-virus interaction, Positive selection

SHIFT 02-118

Topic: ***AS19. Host-pathogen interactions***

CHARACTERIZATION OF HUMAN TIBROVIRUS ENVELOPE GLYCOPROTEINS

Lecture Title:

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Background and Aims: Tibroviruses are emerging rhabdoviruses detected in humans, cattle, and arthropods. Four tibroviruses are known to infect humans: Bas-Congo virus (BASV), Ekpoma virus 1 (EKV1), Ekpoma virus 2 (EKV2), and Mundri virus (MUNV). However, most of these viruses are poorly studied. We aimed to characterize human tibrovirus glycoproteins (G), which likely play a pivotal role in viral tropism and pathogenicity.

Methods: We generated vesicular stomatitis Indiana virus (VSIV) pseudotyped with tibrovirus Gs and virus-like particles (VLPs) bearing respective Gs. VLPs with the four tibrovirus Gs were used to produce mouse antisera for antigenicity comparison. We also investigated the ability of tibrovirus Gs to mediate entry into various cells or to use cellular attachment factors involved in infectivity enhancement.

Results: Tibrovirus Gs share some primary and tertiary structures, although their overall amino acid homology is low (29-48%). Multiple potential glycosylation sites were found on the G molecules, and Endo H- and PNGase F-sensitive glycosylation was confirmed. VLPs produced by transient expression of tibrovirus Gs were used as immunogens and induced potent antibody response. Analysis of mouse antisera using respective pseudotyped VSIVs showed limited cross-neutralizing activity. Human DC-SIGN and TIM-1 were found to act as attachment factors for tibrovirus entry into cells, with BASV G showing the highest affinity. The pseudotyped VSIVs infected a wide range of cell lines with a preferential tropism for human-derived cell lines.

Conclusions: Our findings provide fundamental information for a better understanding of the biological properties of human tibroviruses.

Disclosure: No significant relationships.

Keywords: Bas-Congo virus, Hemorrhagic fever, Tibrovirus, Emerging rhabdoviruses

SHIFT 02-119

Topic: ***AS20. Human microbiome and health***

ISOLATION AND IDENTIFICATION OF GUT MICROBES BY LIQUID-LIQUID CO-CULTURE METHOD

Lecture Title:

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Background and Aims: Gut microbes are of critical importance in various industrial fields including drug development; therefore, the isolation, cultivation, and classification of difficult-to-culture microorganisms and novel species are essential for the development of new live biotherapeutic products. We performed liquid-liquid co-cultures to obtain isolates using a previously unreported method.

Methods: Selectively filtered fecal samples were anaerobically co-cultured under the influence of supporting bacteria through a membrane filter. Isolates were tested for characterization and genomic analysis.

Results: Genomic analyses revealed that the isolates were identified as either *Waltera intestinalis* or a novel species closely related to *W. intestinalis*. Filter-treated isolates grew only in the co-culture. Isolates in broth medium had short and thin cells during the early stages of growth, but became longer and thicker with longer incubation times. Therefore, the cells in the early growth stage may have passed through the filter due to their small size and elongated at a later stage. In addition, all isolates had genes involved in sporulation and germination. This suggests that the small spores that passed through the filter germinated under the influence of the metabolites produced by the supporting bacteria.

Conclusions: Short and thin cells or small spores passed through the filter and were isolated by the liquid-liquid co-culture method. It was suggested that these cells and spores were bacterial species of the genus *Waltera* and grew under the influence of co-culture.

Disclosure: No significant relationships.

Keywords: The genus Waltera, human gut, co-culture method, Waltera intestinalis, Genomic analysis

SHIFT 02-120

Topic: ***AS20. Human microbiome and health***

BIOINFORMATIC METABOLIC CHARACTERIZATION OF THE BLOOD MICROBIOME DYSBIOSIS IN PULMONARY SARCOIDOSIS

Lecture Title:

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Background and Aims: Numerous bacterial genera, including Veillonella, Prevotella, Cutibacterium, and Streptococcus, are typical for pulmonary microbiota but also are frequently linked with atypical inflammatory processes. Research investigating the microbiomes from both blood and bronchoalveolar lavage fluid (BAL) in patients with sarcoidosis reveals that an increased abundance of microbes may play a role in the initiation of granulomatous formation. Moreover, previously genes associated with the terpenoid or mevalonate pathway were found to be enriched among bacteria with granuloma formation potential. This study aims to evaluate the influence of microbiome dysbiosis, particularly through disruptions or activation in terpenoid synthesis, on pulmonary sarcoidosis.

Methods: By applying flux balance analysis (FBA) models under both normal and stressed physiological conditions, we analyzed gene interactions and the metabolic capabilities of these microbial communities.

Results: Comparative analysis demonstrated marked differences in the metabolic profiles of microbiomes from sarcoidosis patients compared to healthy individuals. Our findings suggest that alterations in the terpenoid pathway might link microbiome dysbiosis with the development of pulmonary sarcoidosis. Further, potential biochemical triggers of atypical inflammation are identified on the base of taxonomy composition.

Conclusions: This study emphasizes the critical impact of microbial secondary metabolic pathways, especially terpenoid synthesis, in the pathogenesis of pulmonary sarcoidosis and presents potential new targets for diagnosis and therapeutic intervention. **Acknowledgments: This work was supported by the Bulgarian National Science Fund: grant numbers KP-06-DV/10-21.12.2019 and KP-06-H73/5 5.12.2023**

Disclosure: No significant relationships.

Keyword: pulmonary carcinogenesis, microbiome dysbiosis, terpenoid synthesis, flux balance analysis (FBA)

SHIFT 02-121

Topic: *AS20. Human microbiome and health*

THE INTER-BACTERIAL INTERACTION OF TRADITIONAL AND NEXT-GENERATION PROBIOTICS FOR LACTATE HOMEOSTASIS AND THE ENHANCED PRODUCTION OF SHORT-CHAIN FATTY ACIDS

Lecture Title:

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Background and Aims: The human gut hosts numerous ecological niches for microbe-microbe and host-microbe interactions. The gut lactate homeostasis in humans is crucial and relies on bacteria. *Veillonella* spp., gut lactate-utilizing bacteria, and lactate-producing bacteria were frequently co-isolated. First, the interaction between *V. dispar* and three representative phylogenetically distant strains of lactic acid bacteria was investigated.

Methods: The methodologies of measuring bacterial growth, viability, metabolism, and gene level adaptations in bacterial mono-culture and co-culture were applied.

Results: During co-culture of *V. dispar* with *Lactobacillus acidophilus*, two bacteria exhibited both enhanced growth and increased viability. Our results also indicate that *V. dispar* was able to enhance the fermentative capability of *L. acidophilus* by consuming produced lactate and, therefore, to maintain less acidic environment. We further demonstrated the interaction of *Veillonella* spp. with *Bifidobacterium longan subsp. infantis*, which often coexist in healthy infant gut. The results were also shown as mutually beneficial interaction with enhanced bacterial growth. The production of short-chain fatty acids in the above mutualistic cocultures were all significantly enhanced.

Conclusions: The mechanisms of lactate cross-feeding between traditional probiotics and potential next-generation probiotic bacteria were elucidated to provide future understanding of human lactate homeostasis and new directions in next generation probiotics product development.

Disclosure: No significant relationships.

Keywords: Veillonella dispar, lactobacilli, Bifidobacterium longan subsp. infantis, short-chain fatty acids, lactate cross-feeding

SHIFT 02-122

Topic: ***AS20. Human microbiome and health***

LYSATE OF PARABACTEROIDES DISTASONIS REDUCES THE SEVERITY OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Lecture Title:

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Background and Aims: The microbiota influences the immune system and contributes to differences in host susceptibility to inflammatory diseases. Patients with multiple sclerosis have low abundance of *Parabacteroides distasonis* (Pd) in their gut microbiota and live Pd prevent severe forms of experimental autoimmune encephalomyelitis (EAE) in mice. Here, we tested whether a protective effect can be achieved by Pd lysate and whether this administration affects the gut microbiota composition.

Methods: Lysate of Pd was administered orally to C57BL/6 mice in four weekly doses. Subsequently, EAE was induced and after 3 weeks, we compared the neurological impairments with controls and measured the immune profile by flow cytometry. Microbiota composition was analyzed by next-generation sequencing.

Results: Pd lysate significantly delayed and decreased the severity of EAE. It significantly increased the frequency of regulatory T cells in mesenteric lymph nodes, but not in axial lymph nodes.We found that treatment with Pd lysate did not affect microbial diversity or microbial load, but altered the composition of intestinal microbiota, which was still evident after EAE induction. In Pd treated group we observed an increased abundance of the g*enus Anaerostipes* and genus *Alloprevotella* and decreased family *Tanerellaceae* after EAE induction, but not in controls.

Conclusions: Oral administration of Pd lysate delays clinical symptoms and prevents severe forms of EAE by inducing a T regulatory response. Even killed Pd can protect against severe forms of central nervous system inflammation. This work was supported by the Ministry of Health (NU20-04-00077) and the CAS (LQ200202105) and by the MEYS of the Czech Republic (CZ.02.01.01/00/22_008/0004597).

Disclosure: No significant relationships.

Keywords: Inflammation, bacterial lysate, multiple sclerosis, experimental autoimmune encephalomyelitis, Parabacteroides distasonis

SHIFT 02-123

Topic: ***AS20. Human microbiome and health***

URINARY MICROBIOME PROFILING IN BLADDER CANCER PATIENTS: EXPLORING A NOVEL DIAGNOSTIC MARKER

Lecture Title:

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Background and Aims: Recent studies have shown that the human bladder, previously considered sterile, contains microbes even in healthy individuals. This sparked interest in understanding changes in composition of urinary microbiome associated with disease such as bladder cancer. Our goal was to characterize urinary microbiome profiles in bladder cancer patients.

Methods: We recruited 50 subjects, each providing three samples: mid-stream urine, catheterized urine, and bladder mucous. Bacterial communities were analyzed via V3-V4 region of 16S rRNA gene amplification and Illumina MiSeq sequencing. Data was analyzed using QIIME2 pipeline and EzTaxon-e database.

Results: Bladder mucous exhibit markedly distinct microbial diversity compared to urine, and the bladder mucosal microbiome is significantly influenced by kitomes due to its sparse biomass. The groups categorized by sampling methods showed no meaningful difference in beta diversity. However, within each group, a distinction by gender was evident in mid-stream urine (p-value = 0.004995, PERMANOVA). In catheterized urine of male participants, there was no statistical difference in beta diversity based on bladder cancer status. However, both ANCOM and linear regression analyses identified a specific genus within the family *Comamonadaceae* as significantly more abundant in the cancer group. Examination of various clinical parameters within the patient cohort revealed positive correlation between this particular genus and disease severity, indicating propensity for its prevalence. Similar findings were observed in female patients.

Conclusions: We propose the genus within the family *Comamonadaceae*, as a potential diagnostic marker for bladder cancer. This study was supported by the National Research Foundation of Korea grant funded by the Korea government (MSIT) (No. 2022K1A4A8A02077396).

Disclosure: No significant relationships.

Keywords: urine microbiome, bladder cancer, urobiome, biomarker

SHIFT 02-124

Topic: ***AS20. Human microbiome and health***

EXPLAINABLE AI FOR CERVICAL CANCER STAGE IDENTIFICATION THROUGH VAGINAL MICROBIOME ANALYSIS

Lecture Title:

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Background and Aims: Recent advancements highlight the potential of vaginal microbiome for cervical cancer (CC) screening. Dominated by *Lactobacillus* species, it maintains vaginal health and homeostasis. Studies have shown that the increased non-*Lactobacillus* bacteria correlate with CC progression. These discoveries promoted to the idea of using vaginal biomarkers to identify CC stages through vaginal microbiome analysis. While some bacteria commonly associated with CC, they may not capture individual-specific influential bacteria. In this study, machine learning was used to identify CC biomarker using a local explanation technique called Shapley Additive Explanations (SHAP), offering a more personalized approach compared to global explanation methods. Collectively, our aim is to explore the utility of SHAP for analysing vaginal microbiome data and identifying biomarkers for cervical cancer.

Methods: 362 vaginal microbiomes including three CC stages: non-cancer, pre-cancer (Cervical intraepithelial neoplasia, CIN) and cancer samples were collected from NCBI to construct a trained model via SHAP. Following that, our nanopore sequencing data was utilized to test the trained model, evaluating its performance and identifying potential cervical cancer biomarkers.

Results: Principal component analysis (PCA) of the relative abundance data showed no distinct separation between the three CC stages, whereas SHAP analysis revealed three distinct clusters, indicating varying probabilities of CC occurrence. Notably, it successfully identified both pre-cancerous and cancerous patients within our dataset.

Conclusions: SHAP effectively identified significant bacteria across different CC stages, regardless of bacterial relative abundance. This capability facilitates the identification of cervical cancer stages.

Disclosure: No significant relationships.

Keywords: Vaginal microbiome, Cervical cancer, Shapley Additive Explanations (SHAP), Nanopore sequencing, MACHINE LEARNING

SHIFT 02-125

Topic: ***AS20. Human microbiome and health***

KOREAN GUT MICROBIOME BANK (KGMB), SUPPORT OF MICROBIAL RESOURCES FOR GUT MICROBIOME RESEARCH

Lecture Title:

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Background and Aims: The human gut is home to billions of commensal microorganisms, making up a complex and diverse community known as the gut microbiota. Microbiome research has demonstrated that human health and disease risk are likely related by the gut microbiome. To date, many microbiome studies have focused on metagenomic analysis, making follow-up research using microbial resources difficult. Studying the relationship between the gut microbiome and human disease requires studying the properties and functions of various strains that show marked differences between healthy and diseased individuals. Therefore, to study the gut microbiome, it is essential to collect gut microbiota strains.

Methods: The Korea Gut Microbiome Bank (KGMB) project was started to overcome these limitations of microbiome research and conduct gut microbiome research (supported by the National Research Foundation of Korea (NRF, NRF-2016M3A9F3947962) and the Korea Research Institute of Bioscience and Biotechnology (KRIBB) research project). We isolated and preserved anaerobic microorganisms from the feces of healthy Koreans and also performed metagenomic analysis of each feces.

Results: To date, more than 13,000 strains belonging to more than 450 species have been isolated and preserved from more than 800 healthy Koreans. We are now distributing the strains through our website (https://www.kobic.re.kr/kgmb_dist). We also provide the results of metagenome analysis of the gut microbial community and the coding sequences (CDSs) of the whole genome sequence of each isolate to help users select the strains that suits their purpose (https://www.kobic.kr/kgmb).

Conclusions: To date, microbiome research is being conducted using KGMB resources, and industrial revitalization is expected in the future.

Disclosure: No significant relationships.

Keywords: Korea Gut Microbial Bank (KGMB), resource, healthy Koreans

SHIFT 02-126

Topic: ***AS20. Human microbiome and health***

EXPLORING THE PREBIOTIC POTENTIAL OF KOREAN MEDICINAL PLANTS: SKIN BACTERIAL BALANCE AND BIOLOGICAL ACTIVITIES

Lecture Title:

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Background and Aims: The human skin is home to millions of bacteria and fungi that comprise the skin microbiome. The diversity profile of the human skin microbiome appears to be an indicator of healthy skin or the development of skin diseases. Dysbiosis within the skin microbial populations can lead to the emergence and pathological progression of skin diseases. The purpose of this study was to investigate the prebiotic potential of Korean medicinal plants on skin bacterial balance.

Methods: We measured bacterial growth using OD600 and assessed antibacterial properties of five candidates via the disk agar diffusion method. Anti-inflammatory and antioxidant activities were evaluated by inhibiting NO in LPS-induced cells and through DPPH scavenging, respectively.

Results: After screening the prebiotic activity of various herbal extracts, we selected five for further study: *Quercus salicina* (QS), *Mallotus japonicus* (MJ), *Oenothera laciniata* (OL), *Castanopsis sieboldii* (CS), and *Alnus firma* (AF). In experiments to promote the growth of beneficial bacteria for the skin, OL and CS showed the best effect. We confirmed the antibacterial potential against pathogens like *S. aureus* and found that MJ exhibited strong inhibitory effects. Furthermore, all extracts demonstrated potent antioxidant and anti-inflammatory activities, particularly MJ and AF.

Conclusions: These results suggest that these five plant resources have significant prebiotic activity against skin bacterial models, making them potentially useful ingredients for cosmetics.
This research was financially supported by the Ministry of Trade, Industry and Energy, Korea, under the “Regional Innovation Cluster Development Program (R&D, P0025325)” supervised by the Korea Institute for Advancement of Technology (KIAT).

Disclosure: No significant relationships.

Keywords: Prebiotic Potential, Korean Medicinal Plants, Skin microbiome, Antibacterial activity, Anti-inflammatory Properties

SHIFT 02-127

Topic: ***AS20. Human microbiome and health***

UNRAVELLING THE GUT MICROBIOME LANDSCAPE IN POLYCYSTIC OVARY SYNDROME INSIGHTS FROM METAGENOME TO MICROBIOME

Lecture Title:

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Background and Aims: Polycystic ovary syndrome (PCOS) is characterized by symptoms such as irregular menstruation, formation of cysts in ovary i.e. polycystic ovary, hirsutism, and acne etc. PCOS is one of the most common causes of infertility but the exact cause of PCOS is still unknown. Emerging research suggests that the gut microbiome can play a significant role in PCOS development; a relatively less explored area demanding further investigation.

Methods: In present study, metagenomic data (amplicon and shotgun) are analysed to elucidate the dysbiosis patterns and their functional association with different metabolism in PCOS women. The analysis of 16S rRNA amplicon data was conducted using QIIME2 (version 2023.9) software pipeline. Similarly, shotgun analysis (quality trimming by FaQCs tool, contigs were made with Megahit, bin formation using MaxBin2, metaBAT2 and concoct). Taxonomy and functional profile were done after obtaining high quality of bins. Furthermore, data visualization and analysis of both MiSeq and shotgun data were done in R.

Results: Study have shown reduced microbial diversity of beneficial biomarkers like *Akkermansia, Faecalibacterium, Roseburia,* while increased abundance of opportunistic bacteria such as *Fusobacterium, Escherichia/Shigella* etc in the gut of PCOS women. In the MiSeq data, we observed that the PCOS subjects consuming high-fat diet exhibited lower microbial richness and diversity compared to both the normal and PCOS groups. Similarly, administration of probiotics to the PCOS group led to an increase in both diversity and richness, implying the potential therapeutic utility of probiotics in managing PCOS.

Conclusions: This Suggests that PCOS-associated dysbiosis can lead to further complication and metabolic disorders in PCOS-affected women.

Disclosure: No significant relationships.

Keywords: PCOS, Gut Microbes, Metagenome, Microbial Therapeutics, Metabolic diseases

SHIFT 02-128

Topic: ***AS20. Human microbiome and health***

OPTIMIZING DNA EXTRACTION METHODS FOR SKIN MICROBIOTA ANALYSIS FROM PATCHES FROM SUBMARINES EXPOSED TO HIGH-STRESS ENVIRONMENT

Lecture Title:

Marta Nerini[1], Aldo D'Alessandro[1], Francesca Cialdai[2], Monica Monici[2], Stefano Oliva[3], Alessandro Alcibiade[3], Alessandro Papa[3], Massimiliano Marvasi[4]
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Background and Aims: Long-duration space missions expose astronauts to health risks, including chronic diseases and premature aging. These risks are attributed to changes in microbiota, metabolism, and immune responses, heightened by increased endotoxin and inflammatory signals. The NEPTUNE project investigates these changes by comparing the microbiota and neuroendocrine-metabolic-inflammatory profiles of astronauts and submariners before and after their missions. This comparative study focuses on how confinement, isolation, and altered environmental conditions affect health, while distinguishing effects specific to microgravity and cosmic radiation.

Methods: We utilized 3M Medipore adhesive tape to collect skin microbiota samples from submariners before and after missions of varying durations. The DNA extraction process faced challenges due to the tape's adhesive properties and the presence of additives, which often resulted in low DNA yields.

Results: After testing various DNA extraction kits and lysis protocols, we identified the most effective method for recovering microbial DNA from the tapes. The low DNA yields necessitated the use of Nested PCR to amplify the V3-V4 region of 16S rDNA, facilitating the sequencing and comprehensive analysis of bacterial skin communities. Preliminary results from the microbiota analyses are presented, offering insights into the impacts of mission conditions on microbial diversity.

Conclusions: This research provides critical methods for effective bacterial DNA extraction from adhesive tapes, contributing significantly to health management in confined environments like space and submarines. The established methodologies are poised to enhance future microbial studies in astronauts during space missions.

Disclosure: No significant relationships.

Keywords: Skin microbiome, Stress condition, Submariners, DNA extraction, Nested PCR

SHIFT 02-129

Topic: ***AS20. Human microbiome and health***

EFFECT OF ORAL ADMINISTRATION OF BIFIDOBACTERIUM BREVE M-16V ON FACIAL SKIN IN ADULT WOMEN

Lecture Title:

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Background and Aims: The anti-inflammatory and photoprotective effects of Bifidobacteria on skin have been studied, yet the full effects are unknown. The aim of this study is to investigate the effect of oral administration of *Bifidobacterium breve* M-16V on facial skin in adult women.

Methods: ; In a randomized, double-blind, placebo-controlled trial, 120 women aged 30-79 years (59 probiotic, 61 placebo) were administered *Bifidobacterium breve* M-16V (1×10^{10} CFU) or a placebo twice daily for 12 weeks. Facial skin condition was evaluated by the dermatologist and Canfield VISIA® evolution, at baseline, 4, 8, and 12 weeks. Subjective skin and bowel conditions were also assessed by questionnaire.

Results: In the group under 50 years of age, the total VISIA® score at 12 weeks increased in the placebo group (p=0.001), while the probiotic group showed no increase during the study. The brown spots score at 4 weeks decreased in the probiotic group (p=0.001) and the score was lower than that in the placebo group (p=0.011). The pores scores decreased in the probiotic group at 4, 8 and 12 weeks (p=0.012, 0.049, and 0.0045, respectively). Subjective evaluation of bowel movement was better in the probiotic group at 12 weeks (p=0.006). Skin conditions rated by participants improved in both groups. The incidence of adverse events did not differ between the groups. There were no serious adverse events related to *B. breve* M-16V administration.

Conclusions: Oral administration of *B. breve* M-16V may have beneficial effects on facial skin condition by improving brown spots and pores.

Disclosure: This study was funded by Morinaga Milk Industry Co.,Ltd.

Keywords: Probiotics, Bifidobacterium breve, Skin health

SHIFT 02-130

Topic: ***AS20. Human microbiome and health***

MULTIPLE ORAL ANTIBIOTIC THERAPY FOR THE INDUCTION AND MAINTENANCE OF REMISSION IN PATIENTS WITH ULCERATIVE COLITIS

Lecture Title:

Yuriko Nishikawa[1], Toshifumi Ohkusa[1,2], Tomoyoshi Shibuya[3], Taro Osada[3], Kan Uchiyama[2], Nobuhiro Sato[1]
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Background and Aims: Dysbiosis of the gut microbiota has recently been identified as a therapeutic target for ulcerative colitis (UC). We reported the effectiveness of antibiotic combination therapy (ATM) for the induction and maintenance of UC. In this study, we aimed to investigate the long-term effectiveness of ATM therapy in a larger cohort.

Methods: A prospective open-label trial was undertaken with 311 adult UC patients. The combination of oral amoxicillin 500 mg t.i.d., tetracycline 500 mg t.i.d. and metronidazole 250 mg t.i.d. was administered to patients daily for 2–4 weeks in addition to their conventional medication. Clinical assessment was performed using the partial Mayo score at baseline; at treatment completion; and at 3, 6, 9 and 12 months. Endoscopic evaluation was performed using the Mayo endoscopic score at baseline, 3 months and 12 months.

Results: The compliance rate was 95.7%. The response and remission rates were 75.2% and 30.9% at completion, 62.7% and 29.6% at 3 months, 48.2% and 27.7% at 6 months, 37.9% and 24.4% at 9 months, and 35.4% and 24.4% at 12 months, respectively. The most frequent adverse events were diarrhoea and fever. No life-threatening adverse events were observed during the trial.

Conclusions: ATM therapy effectively led to long-term clinical response and remission in patients with active UC symptoms. And no serious side effects were seen in this study.

Disclosure: Department of Microbiota Research, Juntendo University is a joint research course with Morinaga Milk Industry Co. Ltd.

Keywords: ulcerative colitis, antibiotics, ATM therapy

SHIFT 02-131

Topic: ***AS22. Innate immunity as a first-line against viruses, bacteria and fungi***

MICROBIOTA ENCODED FATTY-ACID METABOLISM EXPANDS TUFT CELLS TO PROTECT TISSUES HOMEOSTASIS DURING CLOSTRIDIOIDES DIFFICILE INFECTION IN THE LARGE INTESTINE

Lecture Title:

Tasia Kellogg[1], Simona Ceglia[2], Abigail Zeamer[1], Vanni Bucci[1], Sage Foley[2], Benedikt Mortzfeldt[2], Doyle Ward[1], Shakti Bhattarai[1], Beth Mccormick[2], Andrea Reboldi[1]
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Background and Aims: The intestinal microbiota's metabolic byproducts play a crucial role in regulating host immune function and inter-species ecological dynamics. Succinate, a metabolite produced by the microbiota, drives the differentiation of tuft cells (TCs) in the small intestine, leading to type 2 immunity-dependent protection against parasites. This study investigates the role of succinate in modulating TC dynamics in the large intestine and its relevance to C. difficile pathophysiology and uncovers a three-way relationship between commensal microbes, C. difficile, and host epithelial cells mediated by succinate.

Methods: Selective microbiota depletion experiments, rational supplementation experiments with succinate-producing bacteria, administration of a succinate-deficient bacterial strain, and prophylactic administration of succinate-producing bacteria.

Results: Selective microbiota depletion experiments reveal elevated levels of type 2 cytokines and expansion of TCs in the colon. Rational supplementation experiments with succinate-producing bacteria confirm the microbiome's role in modulating colonic TC abundance and type 2 cytokine induction. Further experiments using a succinate-deficient bacterial strain show reduced type 2 immunity in mice. Prophylactic administration of succinate-producing bacteria reduces C. difficile-induced morbidity and mortality, confirming the protective role of succinate via the TC pathway

Conclusions: Succinate, an intermediary metabolite of short-chain fatty acid production, increases during dysbiosis. The study suggests that host activation of TCs by microbiota-produced succinate in the colon serves as a defense mechanism against invasion by intestinal pathogens, contributing to the maintenance of barrier integrity and homeostasis.

Disclosure: No significant relationships.

Keywords: clostridioides difficile, Microbiome, tuft cells, large intestine, succinate metabolism

SHIFT 02-132

Topic: ***AS22. Innate immunity as a first-line against viruses, bacteria and fungi***

MALASSEZIA FAIL TO INDUCE TNF-ALPHA SECRETION IN HUMAN MONOCYTE-DERIVED DENDRITIC CELLS

Lecture Title:

Nadji Bouchtout[1], Martin Laurence[2]
[1]CRCHUM, Montreal, Canada, [2]Malassezia Foundation, Montreal, Canada

Background and Aims: *Malassezia* species have emerged as significant players in human health. Metagenomic advancements have uncovered their presence in various organs, linking them to conditions ranging from multiple sclerosis to gastrointestinal disorders. Recent studies, notably by Limon et al. (2019), have highlighted *Malassezia*'s capacity to trigger significant TNF-α production in monocyte-derived dendritic cells, suggesting profound implications for immune responses and disease development. Our aim was to assess the immune response of myeloid cells to *Malassezia, Saccharomyces* and *Candida*, anticipating a robust reaction to *Malassezia* as compared to other fungi.

Methods: Undifferentiated monocytes, monocyte-derived dendritic cells, and GM-CSF-primed monocytes from twelve healthy donors were exposed for 24 hours to whole cells of *Malassezia restricta* (CBS7877), *Malassezia globosa* (CBS7966), *Malassezia furfur* (CBS1878), *Saccharomyces cerevisiae* (BY4741) and *Candida albicans* (ATCC10231), as well as lipopolysaccharide (LPS) and zymosan. TNF-α secretion was quantified by ELISA.

Results: Contrary to our expectations, monocyte-derived dendritic cells exhibited minimal TNF-α production in response to *Malassezia* cells, while significant responses to *Saccharomyces cerevisiae* were observed. Monocytes showed no TNF-α response to *Malassezia*. However, GM-CSF-primed monocytes exhibited a significant TNF-α response to *Malassezia*, indicating context-dependent immune reactions.

Conclusions: In summary, our study challenges the assumption that *Malassezia* is a potent inducer of TNF-α inflammatory responses in human monocyte-derived dendritic cells. Further research is needed to fully elucidate *Malassezia*'s role in disease pathogenesis.

Disclosure: No significant relationships.

Keywords: Malassezia, Dendritic cell, Innate immunity, Crohn's disease

SHIFT 02-133

Topic: ***AS23. Mechanisms of replication and assembly***

DEVELOPMENT OF GENETIC TOOLS TO ELUCIDATE THE METABOLIC CONTROL MECHANISMS OF HALOMONAS SP. A020.

Lecture Title:

Mei Hirata, Yoshinao Azuma, Takuma Otani
Kindai university, Kinokawa, Japan

Background and Aims: *Halomonas* species are alkaliphilic and halophilic bacteria that show promise for the industrial production of diverse biochemicals such as PHB and ectoin. However, genetic tools in the metabolism of *Halomonas* remain limited. This study aims to understand the metabolic regulation of *Halomonas*, and we are currently developing genetic tools such as an efficient gene disruption method.

Methods: We used *Halomonas* sp. A020, isolated from ume seasoning wastewater, in this work. Whole genome analysis of the A020 revealed two plasmids. Initially, the *pyrE* gene was targeted as a marker, easily identifying clones with gene disruption using 5-FOA, an antagonistic agent against pyrimidine biosynthesis. Using the two native plasmids, we constructed expression vectors for *cas9* and a guide RNA targeting the *pyrE* gene in the CRISPR/Cas9 system.

Results: Gene transfer to A020 via electroporation was successful with moderate efficiency, and *pyrE*-gene-disrupted mutants were isolated more effectively than mutagenesis by UV irradiation. In attempting to disrupt the second gene alongside *pyrE*, the *phaC* gene in the PHB metabolic pathway was targeted. As a result, no double gene mutants were isolated while single *pyrE*-gene-disrupted ones were done.

Conclusions: RNAseq was conducted to figure out promoters to increase the expression level of the *phaC* guide RNA, and different promoters were tested for double gene disruption. We will present the results of the double gene disruption using the CRISPR/Cas9 system in this meeting of IUMS2024.

Disclosure: No significant relationships.

Keywords: Halomonas, CRISPR/Cas9

SHIFT 02-134

Topic: ***AS24. Microbial biotechnology and applied microbiology***

"TREATMENT OF RED 40 AZO DYE CONTAMINATED INDUSTRIAL EFFLUENT USING WILD STRAINS OF WHITE ROT FUNGI (WRF) "

Lecture Title:

Camila Gomez, Luisa Munera, Lida Arias Marin
Universidad de Antioquia, Antioquia, Medellin, Colombia

Background and Aims: The azoic dye Red 40 (R40) is widely used in textile and food industries, releasing R40-contaminated effluents into water bodies with demonstrated ecotoxicological effects, highlighting the importance of proper disposal or treatment. Typically, treatment of colored wastewater involves physical methods (adsorption, filtration) and chemical methods (oxidation, coagulation), which often incur high investment costs and may not be highly effective in dye removal. This study evaluates a biological strategy for R40 dye removal in a 6L static bioreactor.

Methods: Fungal strains were obtained through bioprospecting, yielding 7 WRF strains. Their potential for dye transformation/degradation was assessed in solid and liquid media, with optimized culture conditions. Extracellular enzyme production (Manganese peroxidase and Laccase) was determined. The treated effluent was analyzed using nuclear magnetic resonance (NMR) to determine dye transformation/degradation, and a phytotoxicity assay evaluated post-treatment effluent toxicity.

Results: *Lentinus* sp., was chosen from the 7 isolated WRF for its decolorization percentages during assays. In static bioreactor (6L) treatment, 52% degradation was achieved over 12 days from an initial concentration of 80 ppm of dye, with manganese peroxidase enzyme concentration of 120.9 U.min/L at the beginning of degradation process, and effluent toxicity reduced by 75%. RMN data suggests R40 degradation processes by WRF, due to the loss of aromaticity of the parental molecule.

Conclusions: These results highlight a potential way to remove harmful molecules from the environment.

Disclosure: No significant relationships.

Keywords: White rot fungus, Colored effluent, Wastewater treatment, Red 40, azoic dyes

SHIFT 02-135

Topic: ***AS24. Microbial biotechnology and applied microbiology***

OCCURENCE OF PSEUDOMONADS IN A PUBLIC WATER POOL

Lecture Title:

Natalia Gurgacz[1], Karolina Widoniak[1], Maksymilian Mitrowski[1], Marlena Langner[1], Paulina Plewa[1], Mateusz Szukalski[1], Beata Tokarz-Deptuła[1], Adrian Augustyniak[2]
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Background and Aims: Bacteria belonging to the genus *Pseudomonas* are ecologically and biotechnologically relevant microorganisms. Selective pressure at public waterpools caused by the use of various disinfection techniques may result in the occurence of unique strains. Therefore, the aim of the study was to isolate and characterize pseudomonads from a municipal bathing area, taking into account the biotechnological potential of the isolated strains.

Methods: Samples of water, soil and swabs were collected from the swimming pool and surrounding facilities in four sampling campaigns during the summer season (2023) at the Arkonka water pool in Szczecin, Poland. Bacteria were isolated with membrane filtration techniques on selective media. Morphology of colonies and cells were studied under microscope. Gram staining was used to select for Gram-negative bacteria. Biochemical properties were characterized with API20NE assays. Ability to form biofilms was studied with the crystal violet assay. Production of fluorescent pigments was tested by fluorimetry.

Results: In total 409 samples were collected, from which 175 bacterial isolates were obtained. Among them, 33 were characterized by morphology and biochemical features characteristic to the genus *Pseudomonas*. Eighty-six isolates showed the ability to produce at least one pigment, while thirteen strains produced fluorescent substances. Most strains were producing biofilm. Discovered pseudomonads belonged to at least three distinct species.

Conclusions: The study created a diverse collection of microorganisms with potential biotechnological and clinical significance. Microbiological investigations at swimming facilities should address not only water samples, but also areas where there is a risk of bacterial biofilm formation, especially in water-air interfaces.

Disclosure: No significant relationships.

SHIFT 02-136

Topic: ***AS24. Microbial biotechnology and applied microbiology***

BRADYRHIZOBIUM DIAZOEFFICIENS STRAIN WB74 HARBOURS SYMBIOTIC GENES ON GENOMIC ISLANDS (GEI) BUT SHOWS PERSISTENCE IN THE RHIZOSPHERE AND AFFECTS THE ABUNDANCE OF INDIGENOUS RHIZOBIA

Lecture Title:

Ahmed Hassen[1], Khumbudzo Ndlhovu[1], Olubukola Babalola[2], Abe Gerrano[3], Prudence Mtsweni[1], Francina Bopape[1], Francina Bopape[1], Ansa Van Vuuren[1], Elna Van Der Linde[1]
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Background and Aims: *Bradyrhizobium diazoefficiens* strain WB74 is one of the most effective and successful soybean inoculant strains used in the commercial production of soybean in South Africa. Here we report the persistence, in the soybean rhizosphere, of this strain eight years after its last introduction into the soil despite that the rhizobia has all its symbiotic genes located on genomic islands (GEI).

Methods: We investigated the genomic properties with the help of next generation sequencing. The α-diversity of annotated sequence obtained from shotgun metagenomics of the rhizosphere soil sample was estimated from the distribution of the species-level annotations. The soil were used to trap compatible rhizobia in the nodules of soybean in a glasshouse experiment. The annotated genome was used to predict the location of the symbiotic genes using Island viewer4 by aligning it with the reference genome of *B.japonicum* USDA 110.

Results: The analysis of the α-diversity of annotated sequence resulted in 420 species, the majority being *Bradyrhizobium japonicum*. Similarly, the majority of the soil trapped isolates belong to *Bradyrhizobium japonicum* with similar colony morphology and 100% sequence similarity with that of *B. diazoefficiens* strain WB74. Island prediction revealed that all of the symbiotic genes of *B. diazoefficiens* are located on Genomic Islands (GEI).

Conclusions: *B. diazoefficiens* strain WB74 possesses various genetic components required for rhizosphere colonization and inhibition of the growth of other bacterial cells, promoting its own persistence in the rhizosphere. The study provides valuable information in the microbial ecology and interaction of inoculated soybean nodulating *Bradyrhizobium* strains with indigenous soil rhizobia

Disclosure: No significant relationships.

Keyword: Genomic Islands, Persistence, Symbiosis, Bradyrhizobium diazoefficiens, Nodulation, Metagenomics, Ho

SHIFT 02-137

Topic: ***AS24. Microbial biotechnology and applied microbiology***

INTEGRATING A CHIMERIC BINJARI VIRUS NANOTECHNOLOGY INTO PAPER-BASED ASSAYS FOR POC DETECTION OF FLAVIVIRAL INFECTIONS IN VETERINARY APPLICATIONS

Lecture Title:

Jody Hobson-Peters[1], Ryan Johnston[1], Gervais Habarugira[1], Sally Isberg[2], Jessica Harrison[1], Mahali Morgan[1], Lorna Melville[3], Steven Davis[3], Christopher Howard[4], Charles Henry[5], Paul Hick[6], Peter Kirkland[6], Helle Bielefeldt-Ohmann[1], Roy Hall[1]
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Background and Aims: Flaviviruses including Japanese encephalitis (JEV) and West Nile (WNV) viruses have spread globally, adversely affecting people and livestock. When compared to traditional lab-based diagnostics, Lateral Flow Assays (LFAs) offer a simpler, faster, and cost-effective alternative for point-of-care deployment. A chimeric mosquito-specific flavivirus platform, based on Binjari virus (BinJV), was developed to efficiently produce diagnostic nanoparticles for various pathogenic flaviviruses. Using the BinJV genetic backbone, the chimeras are safe and efficient to produce, and antigenically indistinguishable from native pathogenic viruses. BinJV-WNV and BinJV-JEV nanoparticles were paired with LFA for the serological detection of flavivirus infections.

Methods: The novel chimeric BinJV nanotechnology was integrated into a simple, pen-side LFA for WNV and JEV seroconversion detection in susceptible animals (crocodilians and porcines, respectively). The chimeric particles were immobilised onto nitrocellulose membrane as the capture reagent and bound virus-reactive serum antibodies detected with gold nanoparticles conjugated with anti-crocodile IgY or anti-pig IgG.

Results: Using well-characterised sera (n=60) from WNV-seropositive or flavivirus naive Australian saltwater crocodiles, we illustrated 100% sensitivity/specificity, with results achieved in <15 minutes. The LFA further accurately detected seroconversion in animals experimentally infected with WNV. Similarly, the LFA designed for detection of JEV seroconversion in pigs accurately detected JEV exposure of pigs within a farm setting, as well as through experimental infection.

Conclusions: Chimeric BinJV nanoparticles were successfully applied to LFA to produce sensitive and specific pen-side tests for the detection of vector-borne disease in production animals. The assay forms a blueprint for similar point-of-care tests for infection detection in humans and other animals.

Disclosure: No significant relationships.

Keywords: West Nile Virus, Pig, Japanese encephalitis virus, lateral flow assay, crocodile

SHIFT 02-138

Topic: ***AS24. Microbial biotechnology and applied microbiology***

THE TIME-DEPENDENT REACTION OF ANTAGONISTIC STRAIN PRIESTIA MEGATERIUM KW16 ON FUNGAL PHYTOPATHOGENS COLLETOTRICHUM DEMATIUM AND FUSARIUM AVENACEUM

Lecture Title:

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Background and Aims: *Colletotrichum dematium* and *Fusarium avenaceum*, fungal pathogens of many industrial and crop plants, pose a challenging problem in agriculture. Among different methods for controlling these pathogens, applying bacteria with antimicrobial capabilities is the most promising green alternative. Understanding the relationships between biocontrol agents and pathogens is essential to efficient plant disease control. The study aimed to investigate the effect of the pathogenic fungi on the biological activity of the endophytic bacterium *Priestia megaterium* KW16.

Methods: Expression of selected genes was assessed by culturing the KW16 strain in the presence of fungal filtrates. Total RNA was isolated at 24, 48, and 72h and purified prior to cDNA synthesis. The generated cDNA was used as a template in qPCR reactions.

Results: In the presence of both fungal pathogens, significant overexpression of seven tested genes determining direct and indirect biocontrol mechanisms was observed. *F. avenaceum* induced a rapid (after 48h) but a brief bacterial response. Delayed (after 72h), but a several-fold higher response was shown in the presence of *C. dematium*. The fungi triggered in bacteria a distinct upregulation of genes involved in the synthesis of antibiotics (*ppsA*), siderophores (*rhbB*), acetoin (*ilvB*) and the gene encoding oxidative stress enzyme, catalase (*katA*).

Conclusions: The rate and intensity of the response of KW16 to the presence of the fungal pathogen depends on its type, which should be considered when designing an effective biopreparation. This research was funded by the National Science Centre, Poland (grant number UMO-2020/39/B/NZ9/00491).

Disclosure: No significant relationships.

Keyword: biocontrol, endophytes, gene expression, Colletotrichum, Fusarium

SHIFT 02-139

Topic: ***AS24. Microbial biotechnology and applied microbiology***

DECIPHERING THE RELEVANCE OF VISCOSINE IN THE BIOLOGICAL ACTIVITY OF ENDOPHYTIC BACTERIUM OF THE GENUS PSEUDOMONAS

Lecture Title:

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Background and Aims: *Pseudomonas fluorescens* BRZ63 isolated from roots of the oilseed rape inhibited the mycelium growth of *Rhizoctonia solani*, *Fusarium avenaceum*, *Colletotrichum dematium* and *Sclerotinia sclerotiorum*. Analysis of its genome revealed the presence of many genes involved in the production of siderophores, biosurfactants and antimicrobial molecules. The study aimed to determine the role of viscosine in the biological activity of the strain.

Methods: Expression of the *vsm*A gene engaged in viscosine biosynthesis was assessed by RT-qPCR after culturing the bacteria in the presence of fungal filtrates for 24, 48 and 72h. To knock out the gene, a linear cassette composed of a drug-resistance gene flanked by regions homologous to the target gene was obtained and fused with the suicide plasmid pEX18Tc by Gibbson assembly and introduced into bacteria *via* electroporation. Insertional mutants were screened for antagonism against fungal phytopathogens in dual culture assays.

Results: *C. dematium*, *R. solani* and *S. sclerotiorum* induced significant overexpression of *vsmA* after 48h of incubation, while in the presence of *F. avenaceum,* no differences in the gene expression were observed throughout the entire experiment. Based on the nucleotide sequence of the *vsmA* gene, a set of starters was designed, and a drug cassette was obtained for insertional mutagenesis. The antagonism assays showed that the obtained mutants still inhibited fungal pathogens' growth.

Conclusions: Viscosine production is not essential for the antifungal activity of strain BRZ63, which may indicate other mechanisms' involvement in the strain's biological activity. This research was funded by the National Science Centre, Poland (grant number UMO-2020/39/B/NZ9/00491).

Disclosure: No significant relationships.

Keyword: biocontrol, Pseudomonas, lipopeptides, mutagenesis

SHIFT 02-140

Topic: ***AS24. Microbial biotechnology and applied microbiology***

EXPLORING THE INTERPLAY BETWEEN ENDOPHYTIC BACTERIUM PRIESTIA MEGATERIUM KW16 AND RIZOCTONIA SOLANI, A COMMON FUNGAL PATHOGEN OF OIL SEED RAPE

Lecture Title:

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Background and Aims: Endophytic bacteria inhabiting internal tissues of plants provide continuous protection against pathogens and positively affect the growth, development and plant fitness. Biocontrol of phytopathogens is frequently the result of competition for nutrients and niches and the production of lytic enzymes, antibiotics, and volatile organic compounds. Often, multiple mechanisms and their synergistic effects are involved in these processes. This study aimed to determine the impact of *P. megaterium* KW16 on *R. solani* growth, investigate the ability of strain KW16 to protect oilseed rape against the fungal pathogen and explore the effect of the fungus on the expression of genes potentially involved in biological activity of KW16.

Methods: The antagonistic behaviour of KW16, the effect of volatile and diffusible metabolites, was screened through the dual-culture antagonism assays against *R. solani*. The protective role of the bacterium on oilseed rape was tested in pot cultures. Expression of selected bacterial genes was assessed by RT-qPCR after culturing the bacteria in the presence of fungal filtrates.

Results: KW16 showed significant antifungal activity through the production of diffusible and volatile compounds. In response to *R. solani* filtrates, KW16 showed pronounced overexpression of genes engaged in biofilm formation and siderophore, lipopeptides and acetoin production. The bacterium abolished the negative impact of *R. solani* on oilseed rape growth. Its presence strikingly influenced the length and weight of plant roots.

Conclusions: KW16 holds enormous potential for being used as a biocontrol agent. This research was partly funded by the National Science Centre, Poland (grant number UMO-2020/39/B/NZ9/00491).

Disclosure: No significant relationships.

Keyword: endophytic bacteria, biocontrol, gene expression, fungal phytopathogens

SHIFT 02-141

Topic: ***AS24. Microbial biotechnology and applied microbiology***

SCREENING AND OVEREXPRESSION OF A MICROBIOME-DERIVED BACTERIOCIN

Lecture Title:

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Background and Aims: Bacteriocins are gene-encoded, antimicrobial peptides produced by bacteria. They have the potential for development as antibiotic alternatives and are capable of killing antibiotic-resistant pathogens. Human microbiomes have proven to be rich sources of bacteriocin-producing strains. However, one of the challenges of bringing bacteriocins to clinical efficacy is their often limited production from the native producer. Our aim is to mine microbiomes for bacteriocin producers and overcome the limited bacteriocin production through different routes: (1) optimizing the fermentation conditions of the native producer and (2) through heterologous over-expression of the genes involved in peptide production.

Methods: Screening of microbiomes was verified by overlays, spot and well-diffusion assays against the acid-resistant indicator *Lactobacillus delbrueckii ssp. Bulgaricus*. The novel candidates were sequenced using Illumina's MiSeq platform. To optimize the production of bacteriocin from its native producer, response surface methodology (RSM) will be employed. For heterologous over-production, Gibson assembly cloning methods are underway.

Results: Separate screenings of human skin and vaginal microbiomes resulted in the isolation of antimicrobial-producing isolates. The novel bacteriocin producers, identified as coagulase-negative *Staphylococcus hominis*, were found to encode the bacteriocin homicin, a narrow-spectrum, three-component lantibiotic. Homicin inhibits *Streptococcus agalactiae* (neonatal infection) and *Corynebacterium xerosis* (pneumonia and sepsis). RSM will be applied to strategically improve the production of homicin and each homicin peptide will be cloned into separate strains to overcome the rate-limiting step of inadequate immunity.

Conclusions: Given the clinical importance of the target pathogens of the bacteriocin, this novel bacteriocin may have potential as a targeted biotherapeutic in a clinical setting.

Disclosure: No significant relationships.

Keywords: Optimization, Heterologous expression, screening microbiome, bacteriocin peptides

SHIFT 02-142

Topic: ***AS24. Microbial biotechnology and applied microbiology***

DEVELOPMENT OF A METHOD OF DYNAMIC DETERMINATION OF OXIDATIVE STRESS GENERATED IN M. TUBERCULOSIS

Lecture Title:

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Background and Aims: According to the WHO report, 10.6 million people contracted tuberculosis globally in 2021. Out of all
bacterial diseases, tuberculosis has the highest mortality rate. Mycobacterium tuberculosis, especially
Multi-Drug Resistant (MDR) and Extended Drug Resistant (XDR) strains, create severe epidemiological
problems.

Methods: Based on the cellular antioxidant activity (CAA) assay for assessing the antioxidant properties of
foods and dietary supplements, we developed a method to determine oxidative stress in M. tuberculosis as a potential mechanism of action of substances and compounds with antimycobacterial properties. Bacterial cells were incubated with 2',7'-dichlorofluorescein diacetate
(DCFH-DA). Inside the cell, esterases transform DCFH-DA into 2',7'-Dichlorofluorescin (DCFH), which,
in the presence of the reactive oxygen species (ROS), is oxidated to fluorescent 2',7'-dichlorofluorescein (DCF). Artificial oxidative stress was generated with 2,2'-azobis (2-amido propane) dichloride (ABAP). The total fluorescence caused by natural ROS produced by a respiratory
chain was subtracted from that artificially generated by ABAP.

Results: The obtained normalised RFU can be compared with results received analogically for tested compounds.
In this work, we measured and optimised all parameters of these reactions, including time, temperature,
total volume, and concentrations of DCFH-DA, ABAP and DMSO.

Conclusions: We developed a simple, reliable and reproducible method for dynamic determination of oxidative
stress levels generated in M. tuberculosis. The procedure can be used to screen the stressing potential of antimycobacterial substances and compounds. **Acknowledgements:** This work was funded by the Ministry of Education and Science in Poland within
the statutory activity of the Medical University of Lublin (DS 5/2023 and DS 28/2023)

Disclosure: No significant relationships.

Keywords: Tuberculosis, oxidative stress, antimycobacterial activity

SHIFT 02-143

Topic: ***AS24. Microbial biotechnology and applied microbiology***

ISOLATION AND CHARACTERIZATION OF SEAWEED-DERIVED ALGINATE-DEGRADING BACTERIA: IMPLICATIONS FOR PLANT GROWTH ENHANCEMENT

Lecture Title:

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Background and Aims: Alginate is a major component of seaweed and alginate oligosaccharide has been discovered as a natural product for promoting plant growth. To combine a biofertilizer with the seaweed-derived fertilizer, plant growth-promoting bacteria (PGPB) harboring the activity of alginate degradation were aimed to isolate from the Southern seaside area of South Korea, where is the major seaweed producing area.

Methods: Alginate-degrading bacteria were isolated through extensive screening using the dinitrosalicyclic acid methods to measure reducing sugars and identified through the 16s rDNA sequencing. The substrate specificity of bacteria to the alginate, poly-mannuronate, and poly-guluronate was characterized, and enzyme secretion characteristics of alginate lyase were validated. To evaluate their potential in promoting plant growth, the biofilm production and auxin biosynthesis of bacteria was tested too. The plant growth promotion of bacteria was quantitatively measured in the *Arabidopsis* vegetative growth.

Results: Thirteen alginate-degrading bacteria including three new subspecies were isolated, identified, and characterized alginate degradation. Four of them, *Bacillus toyonensis* B2-2, *Pseudomonas khazarica* D6-3, *P. benzenivorans* D9-1, and *Stenotrophomonas cyclobalanopsidis* E1-3, showed strong biofilm-producing capacity expecting to have additional function in the plant growth. And three bacteria, *Vibrio algivorus* B1-1, *V. natriengens* C1-2, and *Marinomonas Polaris* C3-3, verified to produce IAA. These bacteria can be developed as a potential biofertilizer as a multifunctional PGPB.

Conclusions: In this study, a comprehensive screening method was adopted to isolate alginate-degrading bacteria. Through this approach, we successfully isolated and characterized a multitude of PGPR that can be utilized with seaweed fertilizer for promoting plant growth.

Disclosure: No significant relationships.

Keywords: Seaweed, Alginate, Alginate-degrading bacteria, Biofilm, Indole-3-acetic acid

SHIFT 02-145

Topic: ***AS24. Microbial biotechnology and applied microbiology***

SCREENING XYLANASE PRODUCING FUNGI FROM DIFFERENT SOIL CLIMATIC NICHES 0F THE SOUTH CAUCASUS

Lecture Title:

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Background and Aims: Xylanases are a group of depolymerizing enzymes used for the hydrolysis of xylan that is a major component of hemicellulose. Xylanases are present in plant cell walls and are produced by different kinds of microorganisms.This study aimed the screening of xylanases producer strains from the collection of microscopic fungi isolated from different soil climatic niches of South Caucasus,determining the optimal composition of nutrient media and cultivation conditions for the further increase xylanase activity of the selected strains.

Methods: Selection of xylanase producing stains was carried out under submerged fermentation in the following liquid medium, in %: soy bean flour -3.0; Na_2HPO_4-1.5; NH_4NO_3- 0.2; KCl - 0.05; $MgSO_4$ -0.015. (pH-4.5). Xylanase activity assay was performed according Bailey et al.(1992).

Results: As a result of the screening eight xylanase producing strains with different degree of xylanase activities were selected.The optimal pH and temperature for these xylanases was found in the range 5.0-6.5 and 50-65^{0}C accordingly. Among the various inorganic and organic nitrogen and carbon sources tested $NaNO_3$ and xylose was the best to induce xylanase activity. Yeast extract and peptone also effectively induce the enzyme synthesis. Maximum values of enzymatic activity were obtained from *Penicillium canescens* AMT 85 (50 U/ml); *Penicillium canescens* B44(43 U/ml) and *Trichoderma viride* X19 (38 U/ml).

Conclusions: Based on the results obtained the xylanase activity of the selected producers increased by more than 45% when grown on the optimized medium as compared to the basic medium. **Acknowledgments**:The study was supported by Shota Rustaveli National Science Foundation of Georgia,Project FR-23-13296

Disclosure: No significant relationships.

Keywords: Xylanase, Screening, activity, fermentation

SHIFT 02-146

Topic: ***AS24. Microbial biotechnology and applied microbiology***

EVALUATION OF GEORGIAN BASIDIOMYCETES FOR EFFECTIVE DEGRADATION OF CHICKEN FEATHER WASTE AND KERATINASE PRODUCTION

Lecture Title:

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Background and Aims: Many keratinized waste is generated worldwide, including 5 million tons of chicken feathers (CF). Their valorization using microorganisms is considered the best biotechnological alternative to their processing. This study aimed to evaluate the keratinolytic potential of basidiomycetes and to gain knowledge about the physiological mechanisms regulating keratinase production.

Methods: Basidiomycetes submerged cultivation was performed in a rotary shaker Innova 44 at 160 rpm and 27°C. Solid-state fermentation (SSF) was carried out in 100 mL flasks containing 4 g of CF pieces moistened with 10 mL of nutrient medium. Keratinolytic activity was assessed by the gravimetric method, while keratinase was by the accumulation of tyrosine, and by using keratin azure.

Results: Among 23 fungal strains, Panus tigrinus, Pleurotus pulmonarius, and Pseudotrametes gibbosa expressed the highest keratinolytic activity in SSF whereas P. gibbosa followed by Coriolopsis gallica in submerged fermentation of CF. The decrease in the dry weight of the substrate was accompanied by a gradual release of soluble amino acids into the medium. The highest yields of keratinase activity of 595 U/mL and 475 U/mL were observed in submerged fermentation of CF by P. gibbosa and P. tigrinus for 7 and 14 days, respectively. Culture filtrates obtained from these cultures showed excellent potential for enzymatic hydrolysis of CF.

Conclusions: This study contributes to our knowledge of the keratinolytic potential of basidiomycetes and opens the door to their use in the valorization of keratinous wastes and industrial processes such as leather processing. Acknowledgment: This work was supported by the SRNSF of Georgia (Grant No.FR-22-18172).

Disclosure: No significant relationships.

Keywords: fermentation, basidiomycetes, chicken feather, keratinase

SHIFT 02-147

Topic: ***AS24. Microbial biotechnology and applied microbiology***

INVESTIGATING THE ANTIOXIDANT AND ANTI-ACETYLCHOLINESTERASE CAPACITIES OF GANODERMA SPECIES

Lecture Title:

Maja Karaman[1], Milena Rašeta[1], Jovana Mišković[1], Nenad Krsmanović[2], Eleonora Čapelja[2], Sanja Berežni[3], Diandra Pintać Šarac[4]
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Background and Aims: *G. lucidum* and *G. applanatum* are popular medicinal mushroom species while *G. pfeifferi* and *G. resinaceum* have not been much analyzed, hence the aim was to assess the antioxidant and anti- A Ch E activity of four *Ganoderma* species originated from northern Serbia.

Methods: Ethanol (EtOH), water (H_2O), and chloroform ($CHCl_3$) extracts were examined by LC-MS/MS and total phenolic content (TP) and total carbohydrate content (TC) by spectrometry.

Results: Five phenolics: two hydroxybenzoic acids, chlorogenic and quinic, and biflavonoid *amentoflavone* were identified, while almost all were found at the highest conc. in EtOH extracts, with 2,5-dihydroxybenzoic acid as dominant (83-1648 µg/g d.w.) and most abundant in *G. applanatum* / *G. pfeifferi* (1648 µg/g d.w./1580 µg/g d.w., respectively). The highest TP was determined in EtOH (205 - 380 mg GAE/g d.w.) being superior in the ABTS assay (108mg TE/g d.w., *G. applanatum*). The highest TC was found in *G. pfeifferi* $CHCl_3$ (1895 GluE/g d.w., 439 mg FruE/g d.w.). DPPH and FRAP activities were prominent in *G. applanatum* H_2O (IC_{50} 4.62 µg/mL and 248 mg AAE/g d.w., respectively), while $CHCl_3$ extracts stood out in the NO assay (IC_{25} 155 - 340 µg/mL). The most effective AChE inhibitors was *G. pfeifferi*, with the highst activity of EtOH/H_2O (IC_{50} 0.32 µg/mL, 0.34 µg/mL). High correlation (r^2 0.75 - 1) between TP and A Ch E inhibition in EtOH was noticed for *G. lucidum* and *G. pfeifferi*.

Conclusions: These results emphasize the potential therapeutic use of *Ganoderma* species, particularly *G. pfeifferi* in combating oxidative stress and neurodegeneration.

Disclosure: Theauthorsgratefully acknowledge the financial support of the Ministry of Science,Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-66/2024-03/ 200125 & 451-03-65/2024-03/200125).

Keywords: antioxidant, Ganoderma, G. pfeifferi, AChE inhibition, phenolics

SHIFT 02-148

Topic: ***AS24. Microbial biotechnology and applied microbiology***

A SIMPLE AND RAPID APPROACH FOR ELUCIDATING METABOLIC PATHWAYS IN BACTERIA USING CELL-PENETRATING PEPTIDE- PEPTIDE NUCLEIC ACID CONJUGATES

Lecture Title:

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Background and Aims: Bacterial mutants are essential for the stable production of numerous natural compounds. To establish such mutants, non-essential enzymes directly effecting the production of the target compound are identified and are genetically inactivated. However, current approaches to identify and evaluate such enzymes are tedious and time-consuming. Here, we employed cell-penetrating peptide-peptide nucleic acid conjugates (CPP-PNA) as a potential tool for rapid identification and evaluation of such enzymes *in vivo*.

Methods: First, CPP-PNA targeting house-keeping genes in the model bacteria, *Synechocystis* sp. PCC6803, were synthesized and permeation efficiency was evaluated via growth inhibition. Next, using pyruvate as the target compound, CPP-PNA was used to inhibit the translation of the D-lactate dehydrogenase enzyme (Ddh) and to evaluate its relevance towards pyruvate accumulation. Expression of Ddh and other intracellular metabolites were quantified using Western blotting and LC-MS respectively.

Results: When house-keeping genes were targeted, growth inhibition was observed within 24-48h upon CPP-PNA addition, suggesting that they efficiently permeated into strain PCC6803. Similarly, the translation of Ddh was also inhibited by CPP-PNA and pyruvate accumulation was observed within 24h. Interestingly, inhibition of Ddh also resulted in the accumulation of D-lactate suggesting that an alternate pathway may be present for D-lactate production in the bacterium.

Conclusions: In summary, we showed that CPP-PNA can be used as a rapid and efficient tool to evaluate the function of enzymes and their relevance to target compound productivity allowing for easier establishment of bacterial mutants. They can also potentially serve as a quick approach to further understand metabolic pathways within bacteria.

Disclosure: No significant relationships.

Keywords: Cell Penetrating Peptides, cyanobacteria, metabolic pathway, peptide nucleic acid

SHIFT 02-149

Topic: ***AS24. Microbial biotechnology and applied microbiology***

MOLECULAR ANALYSIS OF THE MICROBIAL COMMUNITIES IN KAVART ABANDONED MINE TO ELUCIDATE THEIR BIOTECHNOLOGICAL POTENTIAL

Lecture Title:

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Background and Aims: Kavart is an abandoned mine in Armenia, located near Kapan town in Syunik Region.The aim of this study was to characterize biodiversity of the acid mine drainage sample by modern molecular methods. The prevalence of bacteria related to the formation of AMD in samples of water outflows from Kavart abandoned mine was studied.

Methods: The sample was collected from Kavart mine.The concentration and purified extracted DNA in libraries were determined.Sequencing libraries were constructed using NEBNext UltraTM DNA library prep kit for Illumina following the manufacturer’s instructions.

Results: The application of metagenomic, transcriptomic, DGGE allowed more completely revealing the composition of bacterial community in AMD. It was shown that water outflow samples of copper mine Kavart contained the following genera: *Acidithiobacillus, Leptospirillum, Sulfobacillus*, as well as *Acidiphilium* and other heterotrophic iron reducing bacteria.A total of 105,593 and 115,689 bases read were performed. *Acidiphilium cryptum*, *Acidithiobacillus* sp. and *Acidibacter ferrireducens* were determined according to DGGE analysis.A wide variety of genes related to metal resistance, including protein- encoding genes cusABC, Cu^{2+} exporting ATPase-encoding genes copAB, copper resistance protein-encoding genes pcoBCD, and copper tolerance two component regulatory system cusSR were identified in the metagenomes of CuB. Overall, classified and unclassified data verify that vast microbial biodiversity exists in Kavart which largest could not be identified with classical microbiology techniques.

Conclusions: Thus, the study of diversity and dissemination of microorganisms in ore deposits of Armenia and their activity in AMD generation is very important and actual from the point of view of their dangerous effects on the environment.

Disclosure: No significant relationships.

Keywords: microbial diversity, Microbial communities, Acid mine drainage, Leptospirillum, metagenom

SHIFT 02-150

Topic: ***AS24. Microbial biotechnology and applied microbiology***

PRODUCTION OF SPORE-FORMING PROBIOTICS THROUGH SOLID-STATE FERMENTATION OF PLANT RAW MATERIALS

Lecture Title:

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Background and Aims: The use of bacilli as probiotic products is rapidly expanding due to their inherent ability to form endospores with unique viability and tolerance to extreme environmental conditions, as well as biotherapeutic potential demonstrating immune stimulation, antimicrobial activity, and competitive exclusion. Ease of Bacillus spp.production and stability during processing and storage make them a suitable candidate for the commercial
manufacture of novel foods or dietary supplements for human and animal feeds.

Methods: The scaled-up production of probiotics was performed in polypropylene gas-permeable bags.

Results: This study elucidates cultivation conditions determining B. subtilis IMB-73 and B. amyloliquefaciens IMB-79 growth and enhanced spore formation during solid-state fermentation (SSF) of lignocellulosic biomass. Among the tested growth substrates, wheat bran followed by cilantro residue after extraction of essential oils provided the highest yield of B. subtilis IMB-73 spores (5.5-6.9×10^{10}spores/g biomass) while the soybean mill appeared to be a poor growth substrate for spore formation. In the SSF of lignocellulose, both bacteria secreted comparatively low cellulase and high xylanase activities to ensure good growth of the bacterial cultures.

Conclusions: Supplementation of wheat bran-containing medium with an additional inorganic or organic nitrogen source
increased the spore number. Peptone at the concentration of nitrogen of 100mM provided the highest yield of
probiotics. The feasibility of the developed medium and strategy was shown in scaled-up SSF of wheat bran
in polypropylene bags since a yield of 5.5×10^{11}spores per gram of dry biomass was achieved. **Acknowledgments:** The study was supported by the Shota Rustaveli National Science Foundation of Georgia, project AR-22-3166.

Disclosure: No significant relationships.

Keyword: probiotics, spores, solid-state fermentation

SHIFT 02-151

Topic: ***AS24. Microbial biotechnology and applied microbiology***

EXPLORING THE POTENTIAL USE OF FUNGI FOR WASTE CONCRETE RECYCLING VIA MICROBIALLY INDUCED CALCITE PRECIPITATION

Lecture Title:

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Background and Aims: The construction industry, particularly the production of Portland cement, is one of a major contributor to global anthropogenic CO_2 emissions. One such strategy to decrease energy consumption and CO_2 emissions involves the process of microbially induced precipitation of calcium carbonate, where waste concrete fines (WCF) can be reused through the microbial activity of microorganisms, namely the fungus *Trichoderma reesei* DSM 768 and the bacterium *Bacillus cohnii* DSM 6307.

Methods: In this study, we investigated the ability of microbially induced calcite precipitation (MICP) by the fungus *Trichoderma reesei* DSM 768, which was compared with the bacterial strain *Bacillus cohnii* DSM 6307. Different type of waste concrete fines fraction (WCF) and different experimental conditions (calcium source, pH adjustment) were examined. Final analyses include scanning electron microscopy (SEM), X-ray diffraction (XRD) and evaluation of mechanical properties by indentation.

Results: The presence of $CaCO_3$ crystals was detected by SEM analysis in all samples. The crystalographic structure (XRD) was determined by observing calcium carbonate crystals types like calcite, vaterite and aragonite. The optimal source of calcium for *T. reesei* was found to be calcium chloride in contrast of *B. cohnii*, where calcium lactate is used. It was confirmed that the pH-value of concrete significantly affects the precipitation of calcium carbonate crystals.

Conclusions: Based on our findings, the utilization of fungus *T. reesei* DSM 768 and bacterium *B. cohnii* DSM 6307 for MICP presents a promising approach for recycling waste concrete fines, resulting in a more efficient and environmentally friendly concrete recycling process.

Disclosure: Acknowledgement: The project was funded by the Czech Science Foundation project no. 22-02702S and by UCT Internal Grant Agency projects A2_FPBT_2023_021 and A2_FPBT_2024_016.

Keywords: fungi, microbially induced calcite precipitation, Waste Concrete Fines, Carbon Footprint, CaCO3 crystals

SHIFT 02-152

Topic: ***AS24. Microbial biotechnology and applied microbiology***

DISCOVERY OF UNIQUE CROSS-DOMAIN PARASITISM BETWEEN CANDIDATUS PATESCIBACTERIA/CANDIDATE PHYLA RADIATION (CPR) AND METHANOGENIC ARCHAEA

Lecture Title:

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Background and Aims: In the domain *Bacteria*, one of the largest, diverse, and environmentally ubiquitous phylogenetic groups, *Candidatus* Patescibacteria (also known as Candidate Phyla Radiation/CPR), remains poorly characterized, leaving a major knowledge gap in microbial ecology. Here, through combining cultivation and microscopy, we discover that *Ca*. Yanofskyibacteriaceae and *Ca*. Minisyncoccaceae belonging to *Ca*. Paceibacteria (formerly known as *Ca*. Parcubacteria/OD1) can parasitically interact with the domain *Archaea*. Furthermore, we attempted to elucidate the host cell attachment mechanisms through metagenomics/metatranscriptomics.

Methods: Physiological and morphological characteristics of the *Ca*. Paceibacteria–methanogen interactions were confirmed by microscopic observations based on fluorescence in situ hybridization (FISH), transmission electron microscopy (TEM), and scanning electron microscopy (SEM). To confirm their interactions based on gene expression levels, we performed metatranscriptomics for the enrichment cultures on days 14 and 31.

Results: The predominant *Ca*. Patescibacteria were families *Ca*. Yanofskyibacteriaceae and *Ca*. Minisyncoccaceae. *Methanothrix* and *Methanospirillum* cells were attached with *Ca*. Yanofskyibacteriaceae and *Ca*. Minisyncoccaceae, respectively. These cells had significantly lower cellular activity than those of the methanogens without *Ca*. Patescibacteria through FISH-based fluorescence. Based on gene expression and the structural predictions, *Ca*. Yanofskyibacteriaceae and *Ca*. Minisyncoccaceae highly expressed extracellular enzymes with peptidoglycan-binding domains, which might be relevant to the host cell attachment.

Conclusions: We found that the interactions between the class *Ca*. Paceibacteria and methanogenic archaea are parasitism. In addition, we identified highly expressed extracellular proteins containing peptidoglycan-binding domains among the three archaea-parasitizing *Ca*. Paceibacteria.

Disclosure: No significant relationships.

Keywords: Candidate Phyla Radiation (CPR), Candidatus Patescibacteria, cross-domain parasitism, anaerobic ecosystems, Candidatus Paceibacteria (formerly Parcubacteria/OD1)

SHIFT 02-153

Topic: ***AS24. Microbial biotechnology and applied microbiology***

ACTIVE AND STABLE XYLANASES OF MICROSCOPIC FUNGI

Lecture Title:

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Agricultural University of Georgia, Department Of Biotechnology, Tbilisi, Georgia

Background and Aims: Although there are several microbial strains that produce xylanases, only a few fungal strains meet the criteria for industrial application. The main issues with the use of xylanases in industry are the high cost and low stability of the great majority of commercial enzymes, which do not always meet the criteria of industrial processes.

Methods: To evaluate pH and heat stability, the crude enzyme extract obtained by SmF was incubated at different pH buffers (pH 3.0-10.0) and temperatures (40-80°C) for 1 hour without substrate. The remaining enzyme activity was assessed using a standard method.

Results: The thermal and pH stability of three active xylanase producers, *Penicillium canescens* AMT 85 (50 U/mL), *Penicillium canescens* B44 (43 U/mL), and *Trichoderma viride* X19 (38 U/mL), was investigated. Xylanase from *Penicillium canescens* B44 was most active at pH 5.5, with more than 70% of its maximal activity occurring between pH 4.0 and 8.0. After 1 hour of incubation at 70°C, it retained 70% of its initial activity. Xylanase from *Penicillium canescens* AMT 85 preserved more than 60% of its initial activity after 1 hour of incubation at pH 5.5-8.5, and 50% of its relative activity when incubated at 65°C. *Trichoderma viride* X19 activity decreased significantly at pH 3.5 and 8.0, and at 55°C.

Conclusions: The obtained results demonstrated that selected strains produce xylanases that are acceptable for commercial applications due to their excellent thermostability and wide pH range.
Acknowledgments: The study was supported by Shota Rustaveli National Science Foundation of Georgia, Project FR-23-13296

Disclosure: No significant relationships.

Keywords: microscopic fungi, Xylanase, stability

SHIFT 02-154

Topic: ***AS24. Microbial biotechnology and applied microbiology***

ACTINOBACTERIAL BIOFERTILIZERS MODULATE ORIGANUM PLANT GROWTH AND BIOMOLECULE PRODUCTION

Lecture Title:

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Background and Aims: The use of chemical fertilizers in agriculture is causing severe environmental problems. A sustainable alternative to crop fertilization is necessary and microbial-based biofertilizers are a promising opportunity. Actinobacteria, such as *Streptomyces violaceoruber* and *Kocuria rhizophila,* are good candidates for formulations since they act as plant-growth promoting bacteria (PGPB) producing bioactive molecules (i.e., siderophores) that increase nutrient availability and mitigate biotic and abiotic stress resulting in higher crop yield. *S. violaceoruber* and *K. rhizophila* were used in vitro and open field experiments on the Mediterranean aromatic plant Oregano to ascertain the effects on plant-growth and bioactive metabolite production.

Methods: Plantlets were grown in vitro (from seed) and in open field (explants from a Sicilian field). Oregano root colonization by the two PGPB was assessed by SEM. Plant explants were inoculated in pots, transplanted in open field, and monitored until flowering on Soil Plant Analysis Development (SPAD), heights, and daily increments. GC-MS volatilome analyses were performed on flowers and essential oils, obtained by hydro-distillation, were also analyzed by GC-MS.

Results: Significant differences were observed in vitro on germination and root elongation. SPAD was positively influenced by *S. violaceoruber*, while *K. rhizophila* influenced daily increments and plant heights when compared to control plants. GC-MS volatilome analyses performed on flowers revealed that the actinobacterial consortium increased 10-fold the amount of p-cymene when compared to the control. Oregano essential oils showed that inoculation with *K. rhizophila* reduced the terpenoids content.

Conclusions: Actinobacterial inoculum exerts a positive effect on oregano plant-growth parameters and modulates its metabolome.

Disclosure: No significant relationships.

Keywords: Actinobacteria, biofertilizers, plant-microbes interaction, bioactive metabolites

SHIFT 02-155

Topic: ***AS24. Microbial biotechnology and applied microbiology***

A LOW-ENDOTOXIC SALMONELLA DELIVERS IB VIRUS IMMUNOGENS VIA A DUAL-PROMOTER VECTOR SYSTEM THAT DRIVES PROTECTIVE IMMUNE RESPONSES THROUGH MHC CLASS-I AND -II ACTIVATION

Lecture Title:

John Hwa Lee
Jeonbuk National University, College Of Veterinary Medicine, Iksan, Korea, Republic of

Background and Aims: An effective vaccine strategy is indispensable against infectious bronchitis virus (IBV) and fowl typhoid (FT), both of which threaten the poultry industry. This study demonstrates a vector system, pJHL270, designed to express antigens in prokaryotic and eukaryotic cells. The vector system stimulates immune responses via synchronized antigen presentation to MHC class-I and -II molecules to produce balanced Th1/Th2 responses.

Methods: The vaccine antigens were crafted by selecting the consensus sequence of the N-terminal domain of the spike protein (S1-NTD) and a conserved immunogenic region of the nucleocapsid protein ($N_{321\text{-}406\ aa}$) from IBV strains circulating in South Korea. The vaccine antigen was cloned and transformed into a live-attenuated *Salmonella* Gallinarum (SG) strain, JOL2854 (Δ*lon*, Δ*cpxR*, Δr*faL*, Δ*pagL*, Δ*asd*). Western blot analysis confirmed concurrent antigen expression in *Salmonella* and eukaryotic cells.

Results: Oral immunization with the SG-based IBV vaccine construct JOL2918 induced IBV antigen and *Salmonella*-specific humoral and cell-mediated immune responses in chickens. PBMCs collected from immunized chickens revealed that MHC class-I and -II expression had increased 3.3-fold and 2.5-fold, respectively, confirming MHC activation via bilateral antigen expression and presentation. Immunization induced neutralizing antibodies (NAbs) and reduced the viral load by 2-fold and 2.5-fold in the trachea and lungs, respectively. The immunized chickens exhibited multifaceted humoral, mucosal, and cell-mediated responses via parallel MHC class-I and -II activation as proof of a balanced Th1/Th2 immune response.

Conclusions: The level of NAbs, viral load, and gross and histological analyses provide clear evidence that the construct provides protection against IBV and FT.

Disclosure: No significant relationships.

Keywords: Infectious bronchitis virus,, fowl typhoid, MHC class-I and II activations, dual-expression vector system, live-attenuated Salmonella Gallinarum

SHIFT 02-156

Topic: ***AS24. Microbial biotechnology and applied microbiology***

SALMONELLA-DELIVERED COBRA-HA1 ANTIGEN DERIVED FROM H1N1 HEMAGGLUTININ SEQUENCES ELICITS IMMUNITY AND BROAD-SPECTRUM PROTECTION AGAINST INFLUENZA A SUBTYPES

Lecture Title:

John Hwa Lee
Jeonbuk National University, College Of Veterinary Medicine, Iksan, Korea, Republic of

Background and Aims: This study incorporates computationally optimized broadly reactive antigen (COBRA) strategy, which leverages the hemagglutinin globular head portion (HA1) consensus sequence spanning influenza virus evolution from 1918 to 2021.

Methods: Constructs carrying different HA1 regions were delivered via Salmonella-mediated bactofection using a Semliki Forest virus RdRp-dependent eukaryotic expression system, pJHL204. Immunization of mice with the designed constructs elicited a robust immune response; characterized by elevated IgG levels and CD4+ and CD8+ T cells. Furthermore, constructs #1 and #5 elicited CD4+IFN-γ+ and CD8+IFN-γ+ T cells demonstrating skewing the response toward T helper 1 cells.

Results: . Notably, the designed constructs exhibited protective efficacy, reducing viral titers and lung inflammation in mice challenged with diverse influenza A strains.

Conclusions: This innovation heralds a paradigm shift in influenza vaccine design and delivery, showcasing Salmonella-mediated COBRA-HA1 as a highly effective in vivo antigen presentation strategy, offering broad protection and promising potential in combating seasonal H1N1 influenza and potential pandemic threats.

Disclosure: No significant relationships.

Keywords: Influenza A, COBRA, Salmonella, Broad spectral protection, Immunity

SHIFT 02-157

Topic: ***AS24. Microbial biotechnology and applied microbiology***

CAN WE INFLUENCE MICROBIAL INTERACTIONS WITH NANOFIBERS THROUGH MORPHOLOGY ADJUSTMENT?

Lecture Title:

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Background and Aims: Nanofibrous materials (NMs) hold significant potential in medicine and the food industry, necessitating microbiological safety. While conventional antimicrobial functionalization raises concerns about antimicrobial resistance, recent research highlights morphology modification, particularly fiber diameter and surface density, as a potential solution to mitigate microbial risks.

Methods: We electrospun nine nonfunctionalized polyamide (PA) NMs and polycaprolactone (PCL) NMs with varied fiber diameter and surface density, investigating their interaction with bacteria *Staphylococcus aureus* and *Escherichia coli*. We focused on biofilm formation and cell retention, assessing them through CFU enumeration, resazurin staining, and SEM analysis, with polystyrene (PS) and $AgNO_3$-functionalized PA NMs as controls. Data were analyzed using R programming language.

Results: PA and PCL NMs demonstrated lower susceptibility to bacterial colonization compared to PS. High bacterial retention was observed in filtration tests, with all PA NMs and one PCL NM retaining 100% of filtered cells. Our findings emphasize the importance of NM morphology, especially fiber diameter in biofilm formation and bacterial retention. Thin-fiber PA showed similar biofilm inhibition to PA functionalized with 0.1 wt % $AgNO_3$. Fiber diameter affected biofilm formation differently in *S. aureus* and *E. coli* on PCL NMs, influencing biofilm morphology and bacterial retention.

Conclusions: Understanding nanomaterial morphology's impact on microbial interactions could lead to safer nanomaterials for medical and industrial use in the future. By optimizing morphology parameters, we can influence microbial interactions as needed and potentially decrease the need for high concentrations of antimicrobial compounds in NMs. The research was supported by Czech Science Foundation Grant No. 23-05154S.

Disclosure: No significant relationships.

Keywords: nanofibers, Biofilm, retention, microbial interactions

SHIFT 02-158

Topic: ***AS24. Microbial biotechnology and applied microbiology***

NOVEL TICK-BORNE ENCEPHALITIS VIRUS NANO-LUCIFERASE NEUTRALIZATION ASSAY

Lecture Title:

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Background and Aims: The current gold standard assay to determine the antiviral potency of antibodies elicited by Tick-borne encephalitis virus (TBEV), TBEV vaccines and drug candidates is the Plaque Reduction Neutralization Test (PRNT). This assay is labor-intensive, time-consuming (7 days) and has very limited sample-handling capacity. However, if the TBEV is engineered to express a reporter such as the nano-luciferase (nLuc), this assay would become amenable to a rapid (24 hours) and high throughput format, wherein residual infectivity can be quantitatively measured in terms of reduction in reporter gene expression. Here we propose to develop TBEV nLuc neutralization assay and use it to assess the human immune response induced by both TBEV infection and TBEV vaccines. *Our specific aims are:* Construction of nLuc-TBE virus Testing the human serum samples from infected and vaccinated patients in neutralization assay with nLuc-TBE virus.

Methods: To construct nLuc-TBE virus, a codon-optimized gene encoding nLuc was inserted into the previously constructed TBEV infectious clone. To perform neutralization assay, a defined number of luminescent units of the nLuc-TBE virus were separately pre-incubated with serial dilutions of heat-inactivated sera from TBEV-naïve, infected and vaccinated patients. Subsequently, these nLuc-TBE virus-sera mixtures were used to infect Vero E6 cells in 96-well plates. At 24 hours post-infection, nLuc luminescence was quantified using a Hidex plate reader.

Results: The results from the neutralization assay were analyzed using appropriate statistical tests.

Conclusions: The developed nLuc TBEV neutralization assay could be successfully used in diagnostics as well as in viral-host interaction studies, serving as a viable alternative to PRNT.

Disclosure: No significant relationships.

Keywords: Tick-borne encephalitis virus, Nano-luciferase, Neutralization assay

SHIFT 02-160

Topic: ***AS24. Microbial biotechnology and applied microbiology***

WINERIES SURFACE YEASTS: ENZYMATIC ACTIVITIES AND TOLERANCE TO SODIUM CHLORIDE, GLUCOSE, ETHANOL, SULFITE AND COPPER

Lecture Title:

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Background and Aims: Portugal is among the ten largest wine producers worldwide but the knowledge of the yeast diversity from winery surfaces and their tolerance to the winery environment is largely unknown.

Methods: Seventy yeast isolates from the surfaces of three wineries (Portugal) were tested for their ability to tolerate ethanol (5, 12.5, 15, and 20%), glucose (50 and 60%), sodium chloride (10 and 15%), sulfites (3 and 6%) and copper (0.01%). Eight enzymatic activities were also evaluated (β-glucosidase, urease, amylase, carboxymethylcellulase (CMCase), protease, gelatinase, pectinase, and xylanase). All tests were done at 25ºC, and the results checked at 48h and 5 days.

Results: In winery A, 24.1% of the yeasts grew at 60% glucose, followed by wineries B and C with 5.0 and 9.5%, respectively. Regarding tolerance to 20% ethanol in the three wineries, yeast growth varied between 37.9 and 45.0%. By contrast, low percentages (between 10.3 and 0.0%) were obtained for tolerance to 15% NaCl. Regarding sulfites (6%) 14.3-31% of yeasts tolerated it, while 67 to 97% grew well with copper. Yeast isolates exhibited a wide range of enzymatic activities: low CMCase activity (23.8-30%), followed by proteases (19-45%) and urease (24.1-57.1%). The highest activities were recorded for gelatinase (65-100%), pectinase (72.4-100%), and xylanase (85-100%).

Conclusions: Yeast isolates show high diversity in response to the tested compounds and surveyed enzymes, characteristics with potential biotechnological application. RM and CA are grateful for their PhD grants (BI/UTAD/80/2022, BI/UTAD/81/2022), project C644866286-011 PRR, and Portuguese Foundation for Science and Technology (UIDB/04033/2020, UIDB/00690/2020, UIDP/00690/2020, LA/P/0007/2020, UIDB/00616/2020 and UIDP/00616/2020).

Disclosure: No significant relationships.

Keywords: Winery, Biotechnological, enzymes

SHIFT 02-161

Topic: ***AS24. Microbial biotechnology and applied microbiology***

EFFICACY OF UV-C AND DISINFECTANTS IN ELIMINATING YEASTS FROM WINERY SURFACES AND ENVIRONMENTS

Lecture Title:

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Background and Aims: The use of disinfectants/detergents on winery surfaces has become an issue of debate due to their environmental toxicity and the amount of water used. Thus, the use of UV-C radiation can be an alternative to chemical disinfectants. The effect of UV-C (254nm) was compared with three disinfectants (A, B and C) on winery-native yeast isolates.

Methods: Ten yeasts were tested to low concentrations of two disinfectants (A and B) and one detergent (C) to determine the best concentration and the shortest exposure time. The isolates were also tested to different doses of UV-C for the lowest effective dose. All tests were carried out at 25ºC.

Results: Among the three tested products, the most effective was product C, causing the elimination of 70% of microorganisms, followed by products B (50%) and A (40%). The time taken to inactivate the isolates was similar for all products, in the range 1 to 5 minutes. It should be noted that *Cryptococcus* spp. was the most resistant to products A and B. For *Candida* spp. a UV-C dose of 100 mJ/cm^2 was sufficient to inhibit the growth. For *Cryptococcus* spp. and *Trichosporon* spp. the dose was higher (250 mJ/cm^2). Higher doses, of 750 and 864 mJ/cm^2, inhibited the growth of *Metschnikowia* spp. and *Rhodotorula* spp., respectively.

Conclusions: The results suggest that UV-C was more effective than detergent/disinfectants in killing yeasts. RM and CA are grateful for their PhD grants (BI/UTAD/80/2022, BI/UTAD/81/2022), project C644866286-011-PRR, and Portuguese Foundation for Science and Technology (UIDB/04033/2020, UIDB/00690/2020, UIDP/00690/2020, LA/P/0007/2020, UIDB/00616/2020 and UIDP/00616/2020).

Disclosure: No significant relationships.

Keywords: Radiation dose, Detergent, Exposure time, growth inhibition

SHIFT 02-162

Topic: ***AS24. Microbial biotechnology and applied microbiology***

CHARACTERIZATION OF TWO BACTERIA ISOLATES FROM VITIS VINIFERA LEAVES AS POTENTIAL BIOLOGICAL CONTROL AGENTS AGAINST AGRICULTURE-RELEVANT PATHOGENIC FUNGI

Lecture Title:

Camilla Mandorino[1,2], Marco Vendemia[2], Antonella Salerno[2,3], Maria Francesca Cardone[2], Carlo Pazzani[1], Antonio Domenico Marsico[2]
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Background and Aims: Modern agriculture heavily relies on pesticides use, with global usage reaching approximately 3.5 million tons annually. Despite benefits such as reducing financial losses and increasing crop yields, their widespread use raises significant environmental and health issues. In Europe, reports reveal pesticide residues exceeding safe levels in 22% of monitored water bodies and 83% of tested agricultural soils. Also, human exposure to pesticides poses serious health risks, including poisoning, chronic illnesses like cancer, and reproductive and neurological disorders. As the European Union is aiming to reduce pesticide use by 50% by 2030, a pressing need to develop sustainable alternatives to agrochemicals is becoming crucial, one of them represented by Biological Control Agents (BCAs). At CREA-Research Centre Viticulture and Enology, two bacteria isolates collected from *Vitis vinifera* leaves were tested in vitro for their potential antagonistic activity against six agriculture-relevant pathogenic fungi.

Methods: A 10^7 UFC/ml suspension of the tested bacteria isolates was streaked along the diameter of Potato Dextrose Agar plates and agar plugs cut from colonies of the pathogenic fungi were placed at the opposite sides of the streak. Fungal growth rate was measured every two days, and the growth inhibition area was calculated when the fungal growth front reached the edges of the plate.

Results: The two tested bacteria isolates proved themselves able to inhibit the growth of all the tested pathogens compared to the control plates.

Conclusions: Bacteria revealed their promising potential as effective BCAs. Further studies are undergoing to investigate their biocontrol activity against more pathogenic fungi and their versatility.

Disclosure: No significant relationships.

Keywords: pesticides, Bacteria, BCAs, pathogenic fungi

SHIFT 02-164

Topic: ***AS24. Microbial biotechnology and applied microbiology***

GLOEOPHYLLUM ABIETINUM BCC89 CELLULASE AND XYLANASE PRODUCTION IN SOLID-STATE FERMENTATION OF FOOD INDUSTRY WASTES

Lecture Title:

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Agricultural University Of Georgia, Institute Of Microbial Biotechnology, Tbilisi, Georgia

Background and Aims: The demand for polysaccharide-hydrolyzing enzymes (PHEs) for their application in biofuel production, agriculture, food, textile, and other industries is growing. Therefore, the search for new superproducers of PHE with complete cellulase and hemicellulase systems and elucidation of the physiological features of the regulation of the synthesis of these enzymes remain a primary task.

Methods: FPA was measured with Whatman No.1 according to IUPAC recommendations. CMCase and xylanase activities were assayed by mixing the appropriately diluted samples with low-viscosity CMC or xylan from birch wood.

Results: Among the tested fungi, *Gloeophyllum abietinum* BCC89 appeared to be an overproducer of CMCase (162.5 U/g) and FPA (3.0 U/g) in SSF of wheat bran whereas *S. commune* BCC632 was the best producer of xylanase in SSF of soybean mill (71.8 U/g). The residue after extraction of essential oils from cilantro used for the first time as a growth substrate provided comparatively lower yields of enzyme activities. Supplementation of wheat bran-containing medium with an additional nitrogen source enhanced *G. abietinum* BCC89 growth and enzyme activity. Compared to the control medium, KNO_3 followed by peptone more than two-fold increased the fungus cellulase activity.

Conclusions: Scale-up of SSF in polypropylene bags containing 1.5 kg of optimized wheat bran medium resulted in the accumulation of 167 U/g, 71 U/g, and 3.2 U/g of CMCase, xylanase, and FPA, respectively. Thus, *G. abietinum* is an excellent candidate for the on-site production of PHE. **Acknowledgments:** The work was supported by the SRNSFG project AR-22-3166.

Disclosure: No significant relationships.

Keyword: Total cellulolytic activity (FPA), CMCase, xylanase, solid-state fermentation (SSF)

SHIFT 02-165

Topic: ***AS24. Microbial biotechnology and applied microbiology***

CHARACTERIZATION OF PROMISING CHALCONE DERIVATIVES AS ANTI-TUBERCULAR CANDIDATES

Lecture Title:

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Background and Aims: The development of new anti-tuberculosis drugs is necessary and urgent, because of that our group has been studying many natural and synthetic compounds, standing out chalcone derivatives, especially 3,4-methylenedioxychalcone. PtpA and PtpB, two low molecular weight tyrosine phosphatases (LMW-PTPs) secreted by *Mycobacterium tuberculosis* (*Mtb*) in the macrophage leading to the arrest of phagosome maturation, were identified as *Mtb* targets of some chalcone derivatives. In the present study 41 chalcones derivatives were synthetized and investigated for their activity against *Mtb* H37Rv, potential targets, and cytotoxicity in macrophages.

Methods: MIC values were determined by REMA assay and *in silico* screening was performed using PubChem, SEA, and ChemBL databases, followed by molecular docking (Autodock 4.2). Targets were further studied isolating *Mtb* resistant mutants grown on 7H10 agar plates containing different concentrations of the compounds.

Results: Seven compounds that showed the highest activity (MIC_{90} 10-30 μM) and selectivity (CC_{50} > 70 μM) were further investigated, and for some of them PtpA and PtpB were identified *in silico* as potential targets. To confirm their role as targets, *Mtb* overexpressing strains were constructed to test their MIC to the compounds. Moreover, in order to better identify the mechanism of action of these compounds, resistant mutants will be isolated and the identification of the polymorphism that could be responsible for the observed resistance phenotype will be performed by whole genome sequencing (WGS).

Conclusions: In conclusion, promising antitubercular chalcones were idetified, and LMW-PTPs were predicted as targets. These results encourage further studies to their characterization as leads in tuberculosis drug development.

Disclosure: No significant relationships.

Keywords: chalcone, drug discovery, MYCOBACTERIUM TUBERCULOSIS, natural product, tyrosine phosphatase

SHIFT 02-166

Topic: ***AS24. Microbial biotechnology and applied microbiology***

ULTRAHIGH-THROUGHPUT SCREENING OF ENVIRONMENTAL BACTERIA BASED ON PROTEOLYTIC ACTIVITY USING DROPLET-BASED MICROFLUIDICS

Lecture Title:

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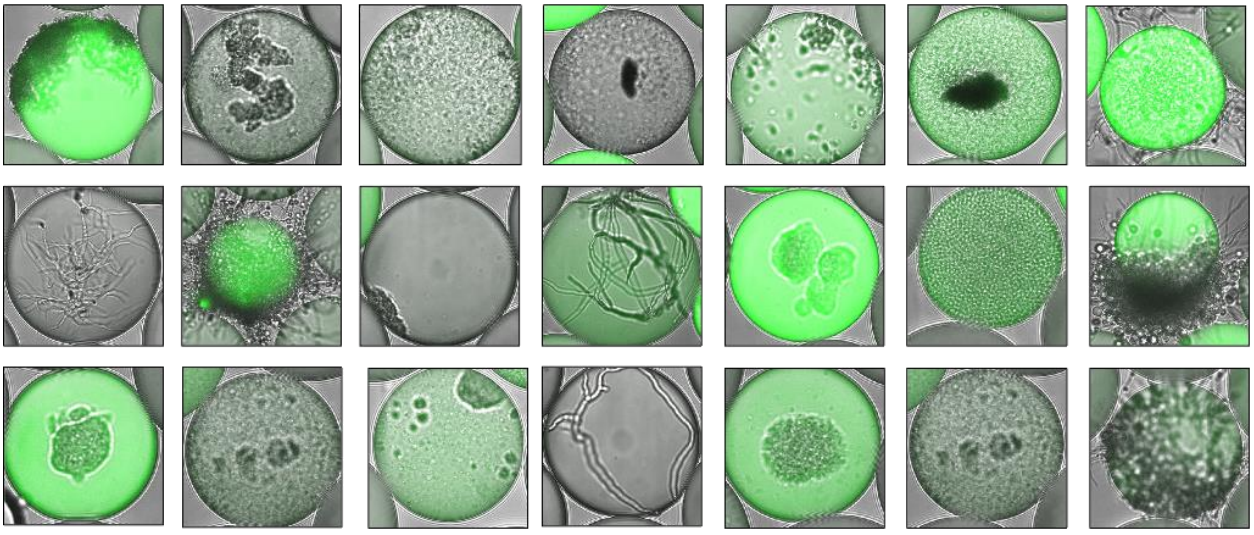
Background and Aims: Environmental bacteria are a vast and underexplored source of enzymes with potential industrial applications. However, it has been suggested that many environmental microorganisms cannot be cultured and isolated by conventional microbial screening methods, due to low throughput. Droplet-based microfluidic systems have been focused on as ultrahigh-throughput microbial screening methods. In this study, we aim to construct a microbial high-throughput screening system targeting endo-peptidase activity.

Methods: Large-scale screening was carried out for soil microorganisms. Perfluorocarbon oil and fluorinated surfactants were used for droplet generation. Droplets were incubated at 30°C for 3 days, and the total analysis and sorting of droplets by fluorescence-activated droplet sorting were 1,002,443 and 10,356, respectively. 94 colonies were randomly selected and isolated based on colony shape.

Results: When environmental microorganisms were cultivated with an indicator to detect endo-peptidase activity, a wide variety of morphologies and peptidase activities were observed (Figure). As a result of the screening, we succeeded in isolating Asp-specific metallo-endopeptidases that showed higher activity than commercially available homologous enzymes used for peptide fingerprinting.



Conclusions: The present study emphasizes the importance and unique potential of microbial screening using the droplet-based microfluidics systems in uncovering novel enzymes from environmental microbes. In particular, the Asp-specific protease obtained in this study, when screened based on endo-peptidase activity, was found to be 2.4 times more active than commercially available related enzymes. The successful isolation of industrially relevant endo-peptidases demonstrates the efficacy of this method, setting the stage for its broader application in industrial microbiology.

Disclosure: No significant relationships.

Keywords: Screening, Protease, Droplet

SHIFT 02-167

Topic: ***AS24. Microbial biotechnology and applied microbiology***

DEVELOPMENT OF A STRAIN-LEVEL MICROBIAL ISOLATION METHOD USING MONOCLONAL ANTIBODIES AND FUNCTIONAL ANALYSIS OF ANTIBODY-TARGETED BACTERIAL MOLECULES

Lecture Title:

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Background and Aims: To maintain health and treat infectious diseases in humans, it is essential to analyze individual gut bacterial strains using simple and accurate methods. The inefficiency of existing cultivation methods, particularly in terms of selectivity for bacterial strains, has led to an increased demand for a method that can efficiently detect, isolate, and cultivate target bacterial strains with high specificity. We hypothesized that generating strain-specific antibodies could be used for the specific detection, isolation, and cultivation of bacterial strains.

Methods: We initiated this study to create a strain-specific monoclonal antibody using Secondary Lymphoid Organ Transplantation (SLOT), which is an *in vivo* application of an antibody production method using artificial lymph nodes, and establish a simple and efficient method for isolating and culturing bacteria from individuals using this antibody.

Results: We first generated an antibody against a human-derived *B. longum* isolate, Jih1, which presented with a competitive advantage when colonizing the intestine. We found that antibody-bound target bacteria could be re-cultured after undergoing magnetic-activated cell sorting. In addition, the target bacteria could be enriched from both an intestinal bacterial mixture and human-derived microbiota that had been supplemented with *B. longum*. The functional analysis of antibodies showed that the target molecule of the antibody was identified as glutamine synthetase (GS). Interestingly, we discovered that Jih1 expressed the normally intracellular enzyme GS on its cell surface, which was specifically recognized by the antibody.

Conclusions: In summary, we developed an efficient bacterial isolation and cultivation method utilizing antibodies against unique, strain-specific molecules.

Disclosure: No significant relationships.

Keywords: ANTİBODY, application, strain-specific antibodies

SHIFT 02-168

Topic: ***AS24. Microbial biotechnology and applied microbiology***

BIOFERTILIZER ON FIELD WHEAT AND ITS EFFECTS ON GROWTH, YIELD, AND NUTRIENT UPTAKE

Lecture Title:

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Background and Aims: Bio fertilizer is a modern form of organic fertilizer that is a selected strain of beneficial soil microorganisms cultivated in the laboratory, including all organic resources used for plant growth, which are provided in a form that can be absorbed by plants through microbial or plant binding or interaction. Our research objectives were to analyze the impact of biological fertilizer treatment on wheat (*Triticum aestivum L*.), to evaluate the effect of biological fertilizer on soil fertility, a wheat yield and quality, treated with IU bio-fertilizer during the wheat cultivation.

Methods: As part of introducing advanced technology, we have collaborated with a research team from Okinawa Agricultural University in Japan to introduce useful microbial technologies to Mongolia. The “Ih Urgats” biological fertilizer, prepared by our research team was a combination of local strains of photosynthetic bacteria, nitrogen fixing bacteria, lactic acid bacteria, yeast, actinomycetes, and fungi.

Results: The current study investigated that the effect of the “Bio-fertilizer”, and the yield of the treated experimental field was 22.4 tons/ha, while the yield of the untreated control field was 14.8 tons/ha, indicating an increase in wheat yield of 7.6 tons/ha.

Conclusions: This study indicates that if biological fertilizers are applied in the field of wheat cultivation and applied to seeds before planting, the benefits of biological fertilizers can be best utilized. The efficacy of IU bio fertilizer, may be increase crop growth and yield can be further enhanced by 7.6 tons/ha, and more.

Disclosure: No significant relationships.

Keyword: Bio+fertilizer+effective+microbes+wheat

SHIFT 02-169

Topic: ***AS24. Microbial biotechnology and applied microbiology***

ISOLATION AND IDENTIFICATION OF BACTERIA WITH POTENTIAL FOR POLYETHYLENE DEGRADATION

Lecture Title:

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Background and Aims: The advantages of polyethylene (PE), which make it one of the most widely used plastics, become its disadvantage when it reaches the natural environment, threatening various organisms and ecosystems. Due to its high hydrophobicity and lack of functional groups, polyethene undergoes prolonged environmental degradation, estimated on a time scale of hundreds of years. Some promising microorganisms have previously been reported to degrade different polyester-type plastics; however, exploring strains that efficiently degrade C-C bonds in polyolefins is still in its infancy. Here, we present a preliminary study in which polyethylene-degrading bacteria were isolated from soils contaminated with plastics.

Methods: In the enriched culture technique, strains with different metabolic properties, synthesizing compounds and enzymes that could potentially facilitate polyethylene degradation that grew on polymer as a sole carbon source were identified based on 16S rRNA sequence analysis. The ability of bacteria to degrade films made of high- (HDPE) and low-density polyethylene (LDPE), and parafilm was further verified by SEM, FTIR and NMR analyses.

Results: Selected strains of the genera *Pseudomonas* and *Bacillus* effectively colonized the entire surface of three polymers. The mechanism of biodegradation depended on the polymer type. For LDPE, mainly loss of branching as well as oxidation of C-C bonds to carbonyl and ester bonds were observed; for HDPE, the formation of internal and terminal C=C double bonds occurred, while for parafilm additionally, a strong hydroxylation of the chains was observed.

Conclusions: Due to different and complementary metabolic features, selected strains can be used to construct microbial functional consortium for enhanced polyethylene degradation.

Disclosure: No significant relationships.

Keyword: bacteria, biodegradation, polyethylene

SHIFT 02-170

Topic: ***AS25. Microbial communities and microbiomes***

MODULATION OF BACTERIAL QUORUM THRESHOLD BY RNA BINDING PROTEIN TOFM IN BURKHOLDERIA GLUMAE

Lecture Title:

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Background and Aims: *Burkholderia glumae,* causing rice panicle blight, employs a LuxR/LuxI(TofR/TofI) type quorum sensing (QS) system. *N*-octanoyl homoserine lactone (C8-HSL) binds TofR, activating the expression of the transcription regulator QsmR. Subsequently, QsmR triggers genes for catalase activity and metabolic slowing, ensuring metabolic homeostasis and survival in crowded conditions. Notably, *B. glumae* exhibits a significantly higher QS onset density under laboratory conditions compared to other QS bacteria. Our aim was to elucidate the molecular mechanisms underlying the abnormally high level of C8-HSL production observed in the *tofM* mutant.

Methods: We employed qRT-PCR, Western blotting, and RNA electrophoretic mobility shift assays to examine TofM-mediated regulation of AHL production and its interaction with *tofI* mRNA. Metabolic effects of the *tofM* mutation were assessed through amino acid quantification, catalase and metabolic activity assays.

Results: We confirmed stringent TofM-mediated QS initiation control and its post-transcriptional regulation of the *tofI* gene, acting as RNA-binding protein. The *tofM* mutant exhibited reduced growth rate attributed to advanced metabolic slowing, evident from higher amino acid levels in its culture supernatants than those of the wild-type. Assessing quorum threshold disruption revealed emergence of non-cooperative *qsmR* mutants from the *tofM* mutant. Additionally, catalase activity was higher in the *tofM* mutant than in the wild-type under high C8-HSL concentrations, while metabolic activity showed a reversed pattern, shedding light on factors driving *qsmR* mutation upon early exposure to high QS signal levels.

Conclusions: Translational control of the QS signal synthase gene at low cell density is a stringent mechanism to maintain metabolic homeostasis and cooperativity in *B. glumae*.

Disclosure: No significant relationships.

Keywords: acyl-homoserine lactone, low cell density, RNA binding protein TofM, cooperativity

SHIFT 02-171

Topic: ***AS25. Microbial communities and microbiomes***

PRELIMINARY METAGENOMIC ANALYSIS OF THE MICROBIAL COMMUNITIES ASSOCIATED WITH CUSHION PLANTS (PLANTAGO RIGIDA) IN THE ECUADORIAN PARAMO.

Lecture Title:

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Background and Aims: Cushion plants are present in almost all high-altitude ecosystems. Their ability to thrive in extreme environments, to act as ecosystem engineers by modulating their surroundings, and their contribution to carbon accumulation and water retention make them crucial components of paramo communities. Certain cushion plant species such as *Plantago rigida* probably undergo a process of micro-succession, where the original plant dies and is replaced by other plant species. Our understanding of this process and the microbial communities involved in it remains limited. Information regarding the microbial communities can elucidate how this process works and the ecological role that microbial groups may play.

Methods: This study analyzed the microbial composition of six samples of *P. rigida* at three stages of plant micro-succession within the Cayambe-Coca National Park – Ecuador. These stages were defined based on the state of colonization of the cushion plant and subsequent new plant species found. Shotgun sequencing was performed to analyze the microbial composition at the different stages. Functional analysis of the genes present in the communities was also performed to understand the possible roles that the microorganisms may have.

Results: Preliminary results revealed very diverse communities that vary in composition between stages, suggesting a succession pattern within the microbial community. *Bradyrhizobium* was the dominant bacterial genus at all stages, while genes associated with nitrogen and carbon fixation as well as photosynthesis were predominant.

Conclusions: These results shed light on the ecological role that these bacterial communities could play in plant micro-succession processes in unique environments such as the Andean paramo.

Disclosure: No significant relationships.

Keywords: metagenomic analysis, Microbial communities, shotgun sequencing, cushion plants, Ecuador

SHIFT 02-172

Topic: ***AS25. Microbial communities and microbiomes***

ORAL-GUT MICROBIOME AXIS INFLUENCE MORTALITY AND DISEASE PROGRESSION IN COLORECTAL CANCER

Lecture Title:

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Background and Aims: Background: The causal factors of Colorectal Cancer is yet not deciphered. Emerging evidences suggests oral-gut axis associated pathogens can translocate to distant sites including colon. Objective: To decipher the CRC tumor-tissue derived microbiota and whether this microbial dysbiosis is linked to periodontal conditions

Methods: Method: 16S rRNA amplicon-based metagenomics method performed to examine 50 matched tumour (CRC)-/adjacent normal (AN) – tissues. Spearman's correlation significance in relation to survival status, TNM stage, microbiome cross-talk, phyla-species evolutionary relationship (Linear Discriminate Analysis Effect Size; LEfSe) and KO/ PICRUSt were performed.

Results: Results: Sequencing data identified *Bacteroides fragilis, Fusobacterium necrophorum, Fusobacterium nucleatum, Veillonella parvula* and *Bacteroides thetaiotaomicron* as unique signature of CRC. Further, Spearman's correlation analysis and ROC curve illustrated the **mortality and disease progression [TNM stages III/IV] predictive power** of *Caldilinea aerophila, Filifactor alocis, Prevotella dentalis, Streptococcus dysgalactiae,* and *Leptotrichia buccalis*. These tumor-derived microbiota are known to be also associated with Periodontitits wherein they support the translocation from oral to intestine. Notably, ***Caldilinea aerophila*** **has not been reported previously in CRC** (83% Specificity, 81% PPV, 71% NPV; p=0.01). Actinomycetales order was found to be over-represented ($p<0.05$) and literature suggests their role in CRC development via TLR4 and NF-kB pathway. Spearman's correlation identified two periodontitis Firmicutes pathogen, *Streptococcus dysgalactiae* and *Staphylococcus epidermidis* association with diet intake (p=0.04).

Conclusions: Conclusion**:** NGS and its analytical tool enabled comprehensive examination of oral-gut microbiome axis, revealing a link between oral microbiome dysbiosis and colorectal cancer. Thus suggesting the potential utility of oral microbiota-based biomarkers for predicting CRC mortality and advancing stage.

Disclosure: No significant relationships.

Keyword: colorectal cancer, dysbiosis, tissue biopsy, tumor tissue microbiome, Lefse analysis, probiotic

SHIFT 02-173

Topic: ***AS25. Microbial communities and microbiomes***

ECOLOGICAL STOCHASTIC PROCESSES IN EXPERIMENTAL MICROBIAL COMMUNITIES

Lecture Title:

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Background and Aims: Stochastic processes such as ecological drift and initial value variability are factors that make the prediction of bacterial community transitions difficult. The relative importance of these processes has been actively debated, but has rarely been verified with actual data. Here, we investigated the influence of stochastic processes by culturing soil-derived bacterial communities in multiple replications under a set of initial conditions and observing their transition.

Methods: We performed the multi-replication (> 40) culture with 8 media conditions and 4 initial community densities for within 2 weeks, and we extracted DNA from these culture samples in times series. DNA was used to 16S rRNA gene amplicon sequencing (515F/806R) to determine the composition of communities, and filtering and classifying the obtained sequence by using bioinformatics pipelines.

Results: In total, 3840 community data (48 replications × 8 media × 6 timepoints + 96 replications × 4 initial community densities × 4 timepoints) were obtained. Under one of the citrate added medium conditions, taxonomic compositions of the replicate communities have been converged. In contrast, under the remaining medium conditions, each replicated community discretely transitioned to several alternative states. The tendency of each replication community to transition to alternative states increased as the initial community density decreased.

Conclusions: By the first case of mass replicated communities, we found that stochastic processes induce discrete transitions (emergence of alternative states). Clarifying the conditions under which the stochastic process makes replicated communities discrete will lead to an understanding of the basis for prediction of bacterial community transitions.

Disclosure: No significant relationships.

Keywords: Community assembly, Stochastic assembly, Multiple stability, Microbiome dynamics, Amplicon sequencing

SHIFT 02-175

Topic: ***AS25. Microbial communities and microbiomes***

INTERSPECIFIC COMPETITION PREVENTS THE PROLIFERATION OF SOCIAL CHEATERS IN AN UNSTRUCTURED ENVIRONMENT

Lecture Title:

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Background and Aims: Bacterial communities are intricate ecosystems in which various members interact, compete for resources, and influence each other's growth. Antibiotics intensify this complexity, posing challenges in maintaining biodiversity. In this study, we delved into the behavior of kin bacterial communities when subjected to antibiotic perturbations, with a particular focus on how interspecific interactions shape these responses.

Methods: Here, we explored how increased interspecific interactions influenced the coexistence of kin bacteria within unstructured and well-mixed habitats in the presence of temporary antibiotic perturbations.

Results: We postulated that a prevalent form of intraspecific interaction among kin bacteria manifested as social cheating under antibiotic stress. In such circumstances, resistant strains effectively removed antibiotics from nearby locations, thereby protecting themselves and neighboring cheaters of the same species. Coexistence became challenging when there were significant differences in the inherent growth rates and competitive abilities between antibiotic-resistant (cooperator) and -sensitive (cheater) strains. To explore potential pathways to coexistence, we incorporated a third bacterial member, anticipating a shift in the dynamics of community coexistence.

Conclusions: Simulations and experimental bacterial communities confirmed our predictions, emphasizing the pivotal role of interspecific competition in promoting coexistence under antibiotic interference. These insights are crucial for understanding bacterial ecosystem stability, interpreting drug-microbiome interactions, and predicting bacterial community adaptations to environmental changes.

Disclosure: No significant relationships.

Keywords: Kin bacteria, Antibiotic impact, Interspecific dynamics, Social cheating, Community coexistence

SHIFT 02-176

Topic: ***AS25. Microbial communities and microbiomes***

PRENATAL DIET SHAPES OFFSPRING MICROBIOME AND METABOLIC HEALTH

Lecture Title:

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Background and Aims: Early-life gut microbiome development plays an important role in the Developmental Origins of Health and Disease (DOHaD). However, the impacts of prenatal Mediterranean- or Western-style dietary patterns on offspring gut microbiome, metabolome, and long-term metabolic and intestinal health remain unclear.

Methods: C57BL/6 mice breeders were given four diets: Western diet with 20% anhydrous milk fat (WD), 20% corn oil (n6), blend of 19% olive oil and 1% fish oil (n3), and Mediterranean diet (MD). Offspring were exposed to these diets exclusively during gestation and nursing, followed by maintenance on a 10-week WD feeding period. Gut microbiome and metabolome were assessed using 16S rRNA sequencing and NMR metabolomics, respectively. Serum lipid profiles were analyzed, and intestinal tissues evaluated for gene expression changes related to inflammation and tight-junction proteins.

Results: Offspring from n3- and MD-fed dams exhibited distinct microbiome beta-diversity compared to WD-fed dams both at weaning and 8-week. Compared to WD, all treatment groups reflected higher abundance of Bacteroidota at weaning and of Bacillota at 8-week. Group-specific genera which maintained higher abundance till 8-week
included *Erypsilotrichaceae* [n3], *Blautia* [n6], and *Intestinomonas* [MD]. Fecal metabolome was modulated considerably in MD group, including higher level of glutamate (weaning) and propionate (8-week) than WD group. The n3 and MD groups had improved lipid metabolism and hepatic function, respectively. Ileal gene expression of claudin-12 decreased, and of IL-10 and IL-17a were relatively higher across all treatment groups compared to WD group.

Conclusions: Prenatal exposure to different dietary patterns distinctly modulates later-life health by shaping early-life gut microbiome development.

Disclosure: No significant relationships.

Keywords: Mediterranean Diet, lipid metabolism, Prenatal nutrition, metabolomics, gut microbiome

SHIFT 02-177

Topic: ***AS25. Microbial communities and microbiomes***

DIVERSITY OF THE NITROGEN FIXING BACTERIA IN TECHNOGENIC SOIL (INOWROCŁAW, POLAND)

Lecture Title:

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Background and Aims: Our previous research found that microorganisms responsible for nitrogen fixation and denitrification are less sensitive to salinity than the nitrifying community in technosoils affected by the soda industry in Inowrocław, Poland. Given the previously undiscovered diversity of nitrogen-fixing bacteria in the region, our study aimed to uncover the diversity of these bacteria.

Methods: To assess bacterial diversity in wheat (*Triticum aestivum* L.) and aster (*Tripolium pannonicum* Jacq.) rhizosphere soil, we employed amplicon sequencing of the *nif*H gene using the Illumina platform. We enhanced nitrogen fixer detection efficiency by using a PVDF membrane for metagenomic DNA extraction with an enrichment step using JMV medium at 26°C for 24 hours. This enrichment step preceded the standard DNA extraction protocol by Qiagen (DNeasy PowerSoil Kit), modified to use PVDF membrane instead of soil.

Results: The diversity of nitrogen-fixing bacteria was higher in the wheat rhizosphere compared to the aster rhizosphere. In wheat, the predominant genus was *Insolitispirillum* (38.80%), followed by unclassified genera within Gammaproteobacteria (9.76%) and *Rhodospirillaceae* (4.74%). Aster rhizosphere was predominantly occupied by *Azotobacter* (95.69%).

Conclusions: The PVDF membrane enabled deeper insights into the rhizosphere bacterial community, detecting more unique amplicon sequence variants (ASVs) compared to the standard protocol. **Acknowledgements**. This study received funding from the European Union's Horizon 2020 Research and Innovation Programme (Grant agreement 101038072) and from EF OBSIDIAN Soil Science, Microbiology, Agricultural Genetics, and Food Quality https://soil-micro.umk.

Disclosure: No significant relationships.

Keywords: Salt stress, Nitrogen fixing bacteria, NGS

SHIFT 02-178

Topic: ***AS25. Microbial communities and microbiomes***

TOWARDS UNDERSTANDING BACTERIAL SENSING AND RESPONSE IN XENORHABDUS GRIFFINIAE TO THEIR ENTOMOPATHOGENIC NEMATODE HOST

Lecture Title:

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Background and Aims: The mutualistic symbiosis between *Xenorhabdus* bacteria and *Steinernema* nematodes has both ecological and practical importance. Their relationship co-evolved to make them dependent on each other in their life cycle where together they kill, feed and reproduce within insect larvae. This behavior has shed light on them as promising candidates to replace conventional pesticides. Despite the importance of *Xenorhabdus* in insect-killing, the mechanisms by which these bacteria might sense and respond to the presence of their nematode host are not well understood. Deeper understanding of these mechanisms can help enhance nematode effectiveness in agricultural applications. Using the entomopathogenic nematode *S. hermaphroditum* and its symbiotic bacterium *X. griffiniae*, we investigated the following aspects: 1) the ability of bacteria to sense nematode-secreted molecules; 2) the functions of bacterial genes involved in nematode-sensing.

Methods: We performed an RNA-sequencing experiment on bacteria in close proximity to their host nematode to identify differentially regulated genes in these conditions, followed by a bacterial genetics study to determine their functional significance.

Results: Secretes from axenic (non-colonized) nematodes cause a significant transcriptional response in the bacterial population. To characterize the function of a top hit gene, *ymdA*, we constructed a deletion mutant in *X. griffiniae*. In *E. coli*, *ymdA* is involved in biofilm formation and antibiotic tolerance, two traits that we hypothesize are important for nematode colonization and bacterial virulence towards insects.

Conclusions: Bacteria modulate gene expression in response to nematode secretes. Studying bacterial gene function in nematode-sensing will expand our knowledge in host-microbe signaling and guide its application in agriculture.

Disclosure: No significant relationships.

Keywords: Host-microbe, entomopathogenic nematodes, RNA-sequencing, bacterial genetics

SHIFT 02-179

Topic: ***AS25. Microbial communities and microbiomes***

FUNGAL MICROBIOME ASSOCIATED WITH THE INVASIVE INSECT PEST TUTA ABSOLUTA (MEYRICK) (LEPIDOPTERA: GELECHIIDAE)

Lecture Title:

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Background and Aims: Advances in next-generation sequencing in recent years have profoundly impacted developments in precision agriculture. The fungal microbiome of the invasive insect pest Tuta absoluta (the tomato pinworm), which causes significant losses in tomato farms, have not been well explored for potential bio-control implementation. In this study, we sequenced and analysed the fungal biota (ITS2 region) associated with larvae and adults of T. absoluta using Illumina NovaSeq PE250.

Methods: Adult and larval *T. absoluta* were collected from farmers' tomato fields and total genomic DNA was extracted from them using a modified cetyltrimethylammonium bromide (CTAB) protocol and sent for sequencing.

Results: A total of 3021 amplicon sequence variants were identified of which 1066 and 2180 ASVs were obtained from the larvae and adults, respectively. 617 bacterial genera were classified, 243 shared between the adults and larval samples while 117 and 257 genera were unique to the larvae and adults, respectively. The functional analysis revealed the fungal biota to be active as saprotroph, symbiotroph and pathotroph. Sample-based biomarkers were identified using Linear discriminant analysis effect size analyses with the fungal species Cladosporium tenuissimum significant in the adults and Sarocladium zeae together with Pichia kudriavzevii significant in the larvae.

Conclusions: Results from this study are important in the quest for a bio-control strategy for Tuta absoluta. Identification of the core fungal microbiome and their major functional group as well as the shared transitory set of fungi between the larvae and adult will be a basis for future exploration and exploitation of microbial-based biocontrol.

Disclosure: No significant relationships.

SHIFT 02-180

Topic: ***AS25. Microbial communities and microbiomes***

EXPLORATION OF AIRBORNE BACTERIAL COMMUNITIES VIA AEROSOLIZATION PROCESS FROM DIFFERENT TYPES OF PIG FARMINGS

Lecture Title:

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Background and Aims: Although the adverse effects of bioaerosols in swine farms are becoming a growing concern, most previous studies have focused on investigating microbial communities and opportunistic pathogens in swine manure. This leaves a gap in aerosols, which have been identified as a major route of transmission in swine farms. Therefore, this study aimed to provide a more detailed understanding of the distribution of airborne microbial communities via the aerosolization process in pig farming.

Methods: Thirty samples, including aerosols, surfaces, and feces, were collected from six swine farms in South Korea. The microbial communities and potential sources of airborne bacteria were analyzed through 16S rRNA gene sequencing. A correlation analysis was also conducted to understand aerosolization interactions between indoor air quality parameters and bacterial communities.

Results: All alpha-diversity indices of bioaerosol samples were significantly higher than the surface in feces ($p < 0.05$). The study revealed that Firmicutes dominated the samples at the phylum level, accounting for approximately 50% of the bacteria community. *Staphylococcus, Clostridium_sensu_stricto_1*, and *Corynebacterium* genera coexisted on the aerosol and surface samples (40% to 70%), apart from the feces samples. In addition, piglet aerosol and surface samples had a higher correlation with *Brevibacterium* and *Staphylococcus* genera, with positive correlations of air quality parameters, such as PM2.5, PM10, and HCHO. *Staphylococcus* species is an antibiotic-resistant bacteria that poses a potential risk to workers and pigs in swine farms.

Conclusions: This study found that the aerosolization of feces and surfaces can contribute to airborne bacterial communities in swine farms.

Disclosure: No significant relationships.

Keywords: Antibiotic resistance, One Health, Bioaerosol, Swine farm, Airborne bacterial communities

SHIFT 02-181

Topic: ***AS25. Microbial communities and microbiomes***

EFFECTS OF ALTERNATIVE SUBSTRATE ON THE MICROBIAL BIODIVERSITY OF THE CHESTNUT SEEDLINGS' RHIZOSPHERE

Lecture Title:

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Background and Aims: Production of chestnut seedlings is generally carried out in pots with standard peat-based soil. However, this method does not always yield healthy and high-quality plants, leading to considerable difficulties in the rooting phase, which may result in insufficient development of the root system or root symbiosis.

Methods: Seeds were planted into three different types of pots (Large Air-pot - AG, Small Air-pot - AP and Frustum of cone-shaped pot V), each filled with two different substrates: one with the highest percentage of peat (VG), and the other with the highest percentage of chestnut fiber (SFC). After one season growth rhizosphere soil was collected and sieved through a 2 mm sieve to eliminate debris and fine roots before DNA extraction. Metabarcoding sequencing of the fungal internal transcribed spacer (ITS2) and the bacterial V4 region of 16S rDNA was conducted to analyse the difference between the microbial communities in the rhizosphere of plants grown in different conditions.

Results: The fungal communities were significantly influenced by the substrates. Specifically, the alpha-diversity was significantly higher in substrate SFC than in VG. In addition, the beta-diversity was significantly different between the substrates, with a predominance of Agaricomycetes in substrate VG. The composition of bacterial communities, too, varied significantly in the two different substrates, but the richness and evenness remained comparable. Furthermore, pot design did not appear to influence the microbiota on plants' rhizosphere.

Conclusions: Alternative substrates combined with pots designed has been identified as a potential approach to improve root system development and biodiversity in the rhizosphere of chestnut seedlings.

Disclosure: No significant relationships.

Keywords: Rhizosphere, substrates, microbial biodiversity, chestnut

SHIFT 02-182

Topic: ***AS25. Microbial communities and microbiomes***

MICROBIAL COMMUNITIES AND DEEP GEOLOGICAL REPOSITORY OF NUCLEAR WASTE

Lecture Title:

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Background and Aims: Management of nuclear waste is a serious environmental problem with understanding of long-term disposal scenarios being required for its safe storage. Deep geological repositories are multi-barrier systems internationally proposed as one of the safest options to dispose of these hazardous materials. Nuclear waste will be encapsulated in metal containers, surrounded by highly compacted bentonites, and buried deeply within a stable geological formation. Bentonites were selected as a sealing and backfilling material due to their good properties, such as high swelling capacity and low hydraulic conductivity. However, bentonite and groundwater indigenous microorganisms can affect the repository stability by, for example container corrosion, change of geochemical conditions or gas release. In this study, compacted bentonites were incubated under *in situ* repository conditions for 14 months in the Äspö Hard Rock Laboratory (Sweden) during which time they were naturally hydrated by two groundwaters with different age, depth, and geochemistry.

Methods: Post incubation, sulfate- and iron-reducing bacteria were anaerobically enriched from the bentonites, to identify the microbial diversity by culture-dependent and culture-independent methods. From those enrichments, DNA was extracted, and microbial communities and isolates were analyzed by 16S rRNA gene sequencing.

Results: The microbial populations were influenced by the different groundwaters, which were very stable based on their geochemical parameters, and with a higher microbial diversity in the shallower water.

Conclusions: Thus, identifying the microbial processes occurring deep underground will help ensuring the safety of future deep geological repositories.

Disclosure: No significant relationships.

Keywords: microbial diversity, deep geological repository, Microbial community, microbial isolates, sulfate- and iron-reducing bacteria

SHIFT 02-183

Topic: ***AS25. Microbial communities and microbiomes***

FIRST INSIGHTS INTO THE MICROBIOTA OF GYPSUM SUBTERRANEAN ENVIRONMENTS TO STUDY THEIR EVOLUTION AND PROTECTION

Lecture Title:

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Background and Aims: Caves are pristine subterranean environments characterized by the absence of light and low nutrients. Studying the biodiversity and metabolic potential of cave microbiomes can provide information on the evolution and protection of subterranean environments and might result in the discovery of novel microbes and metabolites with biotechnological interest. Up to date, most of the studies conducted on subterranean environments have focused on limestone caves, whereas the microbiology of gypsum caves is still understudied. Within this project (in the framework of PNRR-DM118/2023), we provide a first description of the microbiota colonizing the gypsum caves Tanaccia (included in the UNESCO World Heritage Site "Parco Regionale della Vena del Gesso Romagnola") and Buless (within a sulphur-rich area near Rimini).

Methods: Microbial characterization and abundance analyses were conducted through metabarcoding of the 16S rRNA gene and qPCR. Geochemical analyses aimed at assessing macro- and micro-elements as well as organic matter composition are currently in progress.

Results: Microbial diversity analyses showed that Tanaccia is dominated by *Nitrosococcaceae* and *Pseudonocardiaceae* that are generally associated with oligotrophic environments with low anthropic impact. Conversely, Buless cave is dominated by sulphate reducing and sulphur oxidizing bacteria belonging to the families *Desulfocapsaceae*, *Geobacteraceae*, *Sulfurovaceae* and *Sulfurimonadaceae*. The microbial composition can be related with high presence of inorganic sulphur compounds both in the gypsum substrate and in the rising sulfuric waters.

Conclusions: This work provides a first insight into the geomicrobiology of gypsum caves to envision the role of bacteria in gypsum modification and their use as bioindicators for gypsum subterranean environments protection.

Disclosure: No significant relationships.

Keywords: Subterranean environments, Gypsum, Cave microbiota, 16S rRNA gene metabarcoding

SHIFT 02-184

Topic: ***AS25. Microbial communities and microbiomes***

TAXONOMIC AND FUNCTIONAL PROFILING OF BACTERIAL COMMUNITIES ASSOCIATED WITH THE NEWLY DESCRIBED EARTHWORM SPECIES IN THE PHILIPPINES

Lecture Title:

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Background and Aims: Organic matter decomposition and nutrient cycling by earthworms offer valuable applications in the promotion of plant growth and soil fertility. Such is made possible by vermicomposting, wherein interactions of earthworms with their associated microorganisms may play a significant role. In this study, the microbial interactions and potentials of newly described Philippine earthworm species to be used in vermicomposting were explored.

Methods: The bacterial communities associated with the gut and casts of the newly described *Pheretima losbanosensis*, *Polypheretima jenniferae*, and *Polypheretima manticaoensis*, were characterized using QIIME2 (Quantitative Insights into Microbial Ecology 2) and PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2), in comparison with *Eudrilus eugeniae,* a popular vermicomposting species.

Results: Taxonomic profiling revealed that the most dominant bacterial phylum is Proteobacteria followed by Fusobacteria in *P. losbanosensis*, Bacteroidetes in *P. jenniferae*, and Actinobacteria in *P. manticaoensis*, Bacterial families are dominated by *Fusobacteriaceae*, *Aeromonadaceae*, *Weeksellaceae*, *Bacillaceae*, and *Flavobacteriaceae*. Higher numbers of unique species-annotated amplicon sequence variants and distinct bacterial communities (alpha and beta diversity) are found in the gut and casts of the pheretimoid species. Functional profiling predicted the presence of enzymes involved in nitrogen cycle, including ammonia monooxygenase, nitrite reductase, nitric oxide reductase, nitrous oxide reductase, and nitrogenase, and in phosphorus cycle including alkaline phosphatase, acid phosphatase, phytase, and C-P lyase, to name a few.

Conclusions: Overall, bacterial communities associated with the gut and casts have more diverse taxonomic and functional profiles, hence, the potential utilization of the newly described pheretimoid species in vermicomposting as alternatives to *E. eugeniae*.

Disclosure: No significant relationships.

Keywords: bacterial communities, pheretimoid earthworms, taxonomic profiling, vermicomposting

SHIFT 02-185

Topic: ***AS25. Microbial communities and microbiomes***

ADDRESSING THE COMPLEX INTERACTIONS AMONG THE DINOFLAGELLATE OXYRRHIS MARINA AND ITS MICROBIOME ACROSS ECOLOGICAL, GENOMIC, AND METABOLIC SCALE

Lecture Title:

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Background and Aims: Microorganisms are vital for maintaining ecosystem balance and regulating Earth's processes. They are crucial for addressing global challenges and driving industrial and biotechnological innovations. This research aims to explore bacterial microbiome diversity associated with the heterotrophic dinoflagellate *Oxyrrhis marina*, aiming to understand the complex network of interactions and their potential impacts on ecosystems and biotechnology.

Methods: The experiment design included microorganism isolation, SEM and TEM microscopic, whole genome sequencing, metagenomics, microbiome analysis, metabolic reconstructions, and network analysis. Microbial behaviors and population dynamics were evaluated through co-cultures, chemotaxis, and the innovative development of a porous microplate to establish gradients of secondary metabolites.

Results: The study reveals that *O. marina* hosts a diverse bacterial community with significant genetic and metabolic diversity, highlighting contributions to biogeochemical cycles, hydrocarbon degradation, bioremediation, antimicrobial compounds, and products with potential industrial applications. Notable differences in diversity between free-living microorganisms and those closely associated with the dinoflagellate were found. *O. marina* displayed different chemotactic behaviors towards isolated microorganisms, suggesting food preferences. However, bacterial biofilm formation on *O. marina* and population dynamics supported the presence of multiple types of symbiotic interactions. Without significant carbon and nitrogen sources, microorganisms in axenic cultures could not survive or thrive in synthetic seawater. Nevertheless, co-cultures exhibited a positive mutual dependency, where the dinoflagellate and the bacteria benefit each other. Furthermore, metabolic networks exhibited an active and complex exchange of metabolites, revealing important pathways achievable only through microbial cooperation. .

Conclusions: Symbiotic relationships are more dynamic than previously considered, supporting multilateral dependencies with significant ecosystem-level impacts and potential applications.

Disclosure: No significant relationships.

Keywords: Microbiome, Microbial symbiosis, Metabolic network

SHIFT 02-186

Topic: ***AS25. Microbial communities and microbiomes***

EXPLORING PHYLOSYMBIOSIS AND GUT MICROBIOME COMPOSITION IN TROPICAL REEF FISH FROM THE GREAT BARRIER REEF, AUSTRALIA

Lecture Title:

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Background and Aims: The intricate relationships between marine organisms and their microbiomes play pivotal roles in shaping ecosystem dynamics. This study delved deep into the symbiotic associations between tropical reef fish and their gut microbiota, particularly focusing on phylosymbiosis, the co-evolutionary pattern between hosts and their associated microbial communities.The study investigated phylosymbiosis by examining host-microbiome relatedness in select tropical reef fish lineages from the Great Barrier Reef (GBR), the largest reef system in the world and a marine protected area hosting a vast array of fish species. We aimed to uncover the ecological and evolutionary dynamics driving the acquisition of beneficial bacteria, which are crucial for future precision microbiology applications. By studying the gut microbiomes across 11 phylogenetically diverse reef fish families, we sought to understand the core and unique bacterial constituents and their putative functions across different trophic levels.

Methods: Collaborating with the Australian Institute of Marine Science, we utilized DNA samples from 67 reef fish species and sequenced the bacterial 16S rRNA gene using the Illumina MiSeq platform. The resultant sequencing data were analyzed using bioinformatic pipelines (QIIME 2), and statistical analyses were conducted using PRIMER-E and Rstudio.

Results: The results reveal a significant correlation between fish phylogenetic relatedness and their global microbiome composition, offering valuable insights into the ecological and evolutionary dynamics shaping fish microbiomes in the Great Barrier Reef ecosystem.

Conclusions: This interdisciplinary approach, which integrates microbiology, ecology, and bioinformatics, will advance our understanding of the interplay between reef fish and their microbiomes, with implications for marine conservation and ecosystem management.

Disclosure: No significant relationships.

Keywords: Phylosymbiosis, 16S rRNA sequencing, Marine fish, Great Barrier Reef, gut microbiome

SHIFT 02-187

Topic: ***AS25. Microbial communities and microbiomes***

SORTASE-MEDIATED CAPSULAR POLYSACCHARIDE PRODUCTION FACILITATES INTESTINAL COMPETITIVE FITNESS IN RUMINOCOCCUS GNAVUS

Lecture Title:

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Background and Aims: Gut microbiota is substantially associated with our human health and disease, and elucidating the genes and mechanisms of how the bacterium establishes colonization in the gut represents one of the primary goals in this field. *Ruminococcus gnavus* is a prevalent gut bacterium in the human intestine, which is known to correlate with various diseases, such as inflammatory bowel disease, and allergic diseases. This bacterium has various characteristics in cell surface components, such as capsular polysaccharides (cps), inflammatory polysaccharides, and superantigens. This study focuses on sortase, a transpeptidase enzyme responsible for anchoring proteins to the cell wall.

Methods: Based on the genome sequence, *R. gnavus* ATCC 29149 is suggested to possess eight sortase genes, and in this study, we constructed disruptants of each sortase gene. We assessed phenotypic differences in these mutant strains compared to the wild-type strain.

Results: We found that the *srtB4* mutant displayed drastically decreased cps production among these mutants. Additionally, we identified a putative cps biosynthesis gene cluster close to the *srtB4* locus. We confirmed that the disruption of the gene cluster reduced cps production in *R. gnavus*. We also showed that cps-negative isogenic mutants of *R. gnavus* were quickly eliminated in the germ-free mouse intestine during co-colonization with the wild-type strain.

Conclusions: This highlights that sortase-mediated cps production is crucial in competition during establishing gut colonization for *R. gnavus* ATCC29149. We will also discuss the role of other sortase genes in *R. gnavus*.

Disclosure: No significant relationships.

Keywords: gut colonization, capsular polysaccharide, sortase, Ruminococcus gnavus

SHIFT 02-188

Topic: ***AS26. Microbial evolution and diversity***

POLYAMINES INDUCE AN ADAPTIVE MUTATION THROUGH CONTROLLING INTRACELLULAR ROS LEVELS IN E. COLI K-12 STRAIN

Lecture Title:

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Background and Aims: Adaptive mutation leading to a gain in the capacity of an ability to grow is a model for a mutational escape of the stress-induced nongrowing stages and is a prerequisite for an evolution. Despite the evolutionary significance of a stress-inducible mutagenesis, the signaling molecule and signal transduction pathways controlling an adaptive mutation remain to be deciphered. Polyamines (putrescine, spermidine, and spermine), the ubiquitous amine-containing molecules, have various important physiological roles and are essential for a normal cell growth.

Methods: A polyamine-deficient *speABC* mutant JIL601 and its isogenic polyamine-proficient wild type strain QC2461 were grown in a minimal M9 medium containing 0.4% glucose (M9/glucose) as a carbon source and cultivated.

Results: We suggest that the polyamine-deficient mutant abnormally grew during a definite period, after that, the growth was arrested, and then the growth was resumed as a “two-phase” growth. The interval from the growth arrest to the growth resuming is inversely regulated in a polyamine concentration-dependent manner. The mutant also has an elevated SOS response.

Conclusions: Polyamines can act as a signal not only for provoking an adaptive mutation but also for hastening the speed of generating an adaptive mutation. We discuss that polyamines are required for a normal cell growth in a favorable biosystem, but that they act as signal molecules for a mutational survival in an “unfavorable biosystem,” in which some of the cells of the polyamine-requiring group can survive. Our data show that the strength of the selected stress-induced revertant is strongly correlated with the strength of a selection.

Disclosure: No significant relationships.

Keywords: E.coli, adaptive mutation, polyamine, evolution, stress

SHIFT 02-189

Topic: ***AS26. Microbial evolution and diversity***

DEVELOPMENT OF RAPID AND ACCURATE TYPING METHOD BY FT-IR FOR YEAST STRAINS

Lecture Title:

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Background and Aims: When microbial contamination occurs from raw materials or manufacturing processes, it's useful to specify the source of contamination using strain identification technology for proper microbiological risk assessment. Many strain identification technologies have been reported, but most have issues in practicality, accuracy and reproducibility. It has recently been reported Fourier Transform Infrared Spectroscopy (FT-IR), which is expected to be rapid, shows a high correlation with whole-genome sequencing (WGS) analysis in some bacterial species. On the other hand, the number of reports on yeast strain identification by FT-IR is low. In this study, we consider the FT-IR strain identification method by optimizing pretreatment conditions to improve the accuracy of FT-IR analysis for yeast.

Methods: Phylogenetic analysis using both single nucleotide polymorphisms (SNPs) by the WGS and FT-IR analysis under the FT-IR manufacturer's recommended method were performed for *Wickerhamomyces anomalus*. To improve the accuracy of FT-IR analysis, we examined both the culture conditions and the sample preparation conditions for FT-IR analysis.

Results: The results of FT-IR were less accuracy compared with those of WGS analysis. Here, we found that the best reproducibility and identification accuracy were achieved by performing FT-IR analysis with additional washing of cells after incubation with Sabouraud dextrose broth comparing to WGS analysis. Furthermore, the same analyses were also performed for *Candida albicans* and *Saccharomyces cerevisiae*, resulting in agreeing with each other.

Conclusions: The FT-IR analysis under the method examined in this research matched the results of WGS analysis, and can identify yeast strains quickly and precisely.

Disclosure: No significant relationships.

Keywords: Fourier transform infrared spectroscopy, Saccharomyces cerevisiae, strain typing, Candida albicans, Wickerhamomyces anomalus

SHIFT 02-190

Topic: ***AS26. Microbial evolution and diversity***

STREPTOCOCCUS SUIS SUBSP. HASHIMOTONENSIS SUBSP. NOV.: LANCEFIELD GROUP A ANTIGEN–POSITIVE ORGANISMS ISOLATED FROM HUMAN CLINICAL SPECIMENS AND WILD BOAR ORAL CAVITY SAMPLES

Lecture Title:

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Background and Aims: Three *Streptococcus suis*-like strains positive for Lancefield antigen group A were isolated from human boar bite wounds and the oral cavities of boars in Japan. Since Lancefield group A-positive *S. suis* has not been found so far, we investigated their taxonomic positions.

Methods: We carried out several taxonomic analysis methods, such as phenotypic analysis including serotyping, biochemical reactions using API-ZYM and Rapid-ID32Strep, the phylogenetic analysis based on 16S rRNA and *sod*A gene sequences, in silico DNA-DNA hybridization using whole genome sequence, MALDI-TOF-MS analysis.

Results: Three isolates showed positive for Lancefield group A and capsule serotype 5. On the MALDI-TOF-MS analysis, the isolates were assigned to *S. suis* but showed characteristic signal peaks absent for *S. suis*. Sequence analysis of the 16S rRNA and sodA genes determined that the isolates were similar to *S. suis*; however, they appeared on a phylogenetic sub-branch. The ANIb between our isolates and *S. suis* was 94.75%, which was inconclusive; however, digital DNA-DNA hybridization showed a value of 61.2%. Biochemical reactions, such as acid phosphatase and others, distinguished our isolates from *S. suis*.

Conclusions: After a taxonomic investigation based on phylogenetic, genomic, phenotypic characteristics, and MALDI-TOF-MS signal patterns, our isolated strains represented a new subspecies within the *S. suis*. For this, we propose *Streptococcus suis* subsp. *hashimotonensis* subsp. nov. (type strain is PAGU 2482^T).
This is the first report of *S. suis* isolates carrying Lancefield group A antigen. The antigen is suspected to be related to pathogenicity; our isolates are important for human and veterinary clinical practice.

Disclosure: No significant relationships.

Keywords: Streptococcus suis, Lancefield group A–positive,, Streptococcus suis subsp. hashimotonensis, human clinical practice, Wild boar

SHIFT 02-191

Topic: ***AS26. Microbial evolution and diversity***

DIVERSITY OF LEPTOSPIRA SPP. CARRIED AMONG WILDLIFE IN JAPAN.

Lecture Title:

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Background and Aims: Leptospirosis is a zoonotic infectious disease caused by pathogenic *Leptospira* spp. Cases of canine leptospirosis have been reported mainly in the western Japan, but circulation of *Leptospira* spp. in wildlife remains limited. In this study, molecular and serological surveillance of *Leptospira* spp. were conducted to reveal the prevalence and diversity among wildlife in Japan.

Methods: Kidney and serum samples from 23 species of wildlife were collected from 2013 to 2024. *Leptospira* genes, *lipL32* or *rrs2*, were detected from kidney samples by PCR and anti-leptospiral antibodies against six serogroups were detected by microscopic agglutination test (MAT). The PCR-positive samples were further analyzed by multi-locus sequence typing (MLST).

Results: *Leptospira* genes were positive in wild boars, sika deer, Japanese badgers, black bears, raccoon, red fox, and Japanese marten, from which six genotypes of *L. interrogans* were obtained. In five bat species, new genotypes of *L. interrogans*, *L. kirschneri*, and *L. borgpetersenii* were detected. Anti-leptospiral antibodies were positive in various animals, and their serogroups mainly were Australis and Hebdomadis.

Conclusions: This study demonstrated various wildlife carried various genotypes of *Leptospira* spp. Notably bats carried *L. kirshneri* and *L. borgpetersenii*, which have not been reported in Honshu Island, Japan. Several wildlife was serologically positive for serogroups that are not included in vaccines commonly used in Japan, suggesting a necessity of reconsideration on vaccine formulation. Further analysis is required to assess the risk of their transmission to human and domestic animals.

Disclosure: No significant relationships.

Keywords: Leptospira, Leptospirosis, Zoonotic transmission, Genetic diversity, Wildlife surveillance

SHIFT 02-192

Topic: ***AS26. Microbial evolution and diversity***

PROFILES OF LIGNOCELLULOSE-DECONSTRUCTING ENZYME ACTIVITIES IN WHITE-ROT BASIDIOMYCETES UNDER HEAT SHOCK

Lecture Title:

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Background and Aims: In nature, wood-rotting basidiomycetes (WRB) are constantly subjected to environmental stress factors. Heat shock is one of the main abiotic factors influencing fungal growth and biosynthetic activity. However, the knowledge about the impact of heat stress on WRB enzyme production is limited. This study was focused on the production of enzymes involved in lignocellulose degradation.

Methods: *Cerrena unicolor* and *Schizophylum commune* were cultivated in rotary shaker Innova 44 (New Brunswick, USA) at 160 rpm and 27°C. Heat shock was conducted by immersing the flasks with 3-day fungal cultures in a water bath at 45°C for 15, 30, and 60 min. Immediately after treatment and after 1, 2, 4, and 7 days of cultivation, the cultures were analyzed for carboxymethyl cellulase (CMCase), xylanase, laccase, and peroxidase activities.

Results: Among the two strains of C. unicolor, C. unicolor 300 was more resistant to heat shock with a shorter lag phase in enzyme production. Moreover, treatment for 15, 30, and 60 min resulted in a 31-41% increase in the fungus laccase activity compared with a control culture in the fermentation of mandarin pomace. The same regularities were observed for CMCase activity. The highest yields of cellulase and xylanase were observed when S. commune grown in the Avicel-containing medium was exposed to heat shock for 30 min.

Conclusions: The results obtained in this study indicate that a short-term heat shock is a way to achieve stimulation of enzyme activity secretion in WRB strains. Acknowledgment: This work was supported by the SRNSF of Georgia (Grant No.FR-22-1612).

Disclosure: No significant relationships.

Keywords: submerged fermentation, basidiomycetes, heat shock, cellulase, laccase

SHIFT 02-193

Topic: ***AS26. Microbial evolution and diversity***

BOVINE FECES AS A SOURCE OF MULTIPLE NOVEL SPECIES OF THE GENUS ACINETOBACTER

Lecture Title:

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Background and Aims: *Acinetobacter* is a taxonomically and ecologically heterogeneous bacterial genus, which encompasses 83 validly named species. Despite the progress in the systematics of the genus, its species-level diversity in non-human ecosystems remains poorly understood. This study aimed at assessing the taxonomy of culturable acinetobacters in bovine feces obtained from cattle farms in Czechia in 2022.

Methods: Aliquots of homogenized feces were cultured in aerated liquid minimal medium supplemented with acetate at both 30°C and 44°C. Obtained isolates were preliminarily allocated to *Acinetobacter* with MALDI-TOF MS and dereplicated using DNA macrorestriction. The identification/classification at the species level was based on the combination of *rpoB* gene and MALDI-TOF MS analyses. A subset of strains was subjected to WGS and metabolic and physiological testing.

Results: A total of 284 strains were recovered from 28 samples collected in as many farms. Of these strains, 150 (53%) were allocated to 14 known species, with the predomination of *A. indicus* (n=46), *A. pseudolwoffii* (n=30) and *A. gandensis* (n=18). The remaining 134 (47%) strains were classified as 13 tentative novel species (n=108), each containing 3–24 strains, taxonomically unique singletons (n=7) or strains with unclarified taxonomic status (n=19). As many as 240 and 44 strains were obtained from 30°C and 44°C cultures, respectively, with the latter belonging to *A. baumannii*, three novel taxa and two singletons.

Conclusions: The study revealed high proportion and complexity of unknown *Acinetobacter* taxa in bovine feces, which further illustrates a gap in our knowledge of the taxonomic diversity of the genus.

Disclosure: No significant relationships.

Keywords: Taxonomy, Novel taxa, Phylogenomics, Acinetobacter, Metabolic features

SHIFT 02-194

Topic: ***AS27. Microbiology and big data, analyzing and interpreting large datasets***

AI- CONTROLLED MICROBIAL ENUMERATION SYSTEM

Lecture Title:

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Background and Aims: Nowadays both culture-based and molecular-based methods are used to enumerate and characterize microorganisms. However, it is often necessary to quickly and accurately determine the number of microbial cells to perform research experiments, to evaluate the effectiveness of different disinfection or purification methods, and to describe the number of microorganisms in different environments. Furthermore, under conditions of stress (low nutrient concentrations, presence of oxidants) microorganisms can end up in a viable but non-cultivable state, which limits the scope of methods used in identification and enumeration. One of the solutions is microscopy (light or fluorescent), where microorganisms are observed visually. Currently, such microscopic enumeration is done manually - by recording cells microscopically for a laboratory experiment and then recalculating. The main technical limitations are related to enumeration errors, insufficient enumerated field of view and identification accuracy, especially when enumerated cells are not physically separated. The aim of this research was to develop and construct an automated microbial enumeration system based on epifluorescence microscopy and AI identification tools.

Methods: To achieve the aim, a high intensity laser combination system was produced together with specific dichroic mirrors and integrated into a microscope prototype. Further, sample data input was made via education of neural networks.

Results: The system can independently and reproducibly quantify bacteria in water samples prepared on microscopy slides in a time that is not longer than it is currently performed by a qualified laboratory technician.

Conclusions: Research was funded by ERDF project 5.1.1.2.i.0/1/22/A/CFLA/002 (agreement No 2.4.)

Disclosure: Mr Andis Liepins is employed by private company Apply IT Ltd.

Keywords: microscopy, cell enumeration, rapid vizualization

SHIFT 02-195

Topic: ***AS28. Microbiomes and viromes***

THE ANTI-ADIPOGENIC ACTIVITY BY UNUSUAL PROPIONATE PRODUCTION IN A NOVEL HUMAN GUT BACTERIUM

Lecture Title:

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Background and Aims: In the human gut, the members of family *Lachnospiraceae* have been known as major players, which produce SCFAs during microbial fermentation of dietary or host derived carbohydrates. In this study, we aimed to analyze the physiological and genomic characteristics of a novel propionate-producing bacterium in the family *Lachnospiraceae,* designated as strain AP1^T, and determine its function in the human gut.

Methods: Bacterial isolation and culture: Anaerobic bacteria were isolated from fecal samples in the anaerobic chamber (86% N_2, 7% CO_2, and 7% H_2). **Adipogenesis:** 3T3-L1 cells grown in DMEM supplemented with high glucose, 10 % BCS, 1 % penicillin, and 1 % streptomycin were differentiated with adipogenesis stimulators. On day 8 after differentiation, cells were stained with Oil-Red *O* or used for Western blotting analysis.

Results: A strictly anaerobic bacterium AP1^T was isolated from healthy Korean feces. Altough strain AP1^T produced high concentrations of propionate as the major products during fermentation, the genome of strain AP1^T lacked genes for propionate-producing pathways adopted by most gut bacteria. Further genome analysis showed that AP1^T produced propionate via the amino acid catabolic pathway, which was also supported by increased propionate levels after amino acids supplementation. In addition, cell-free supernatant of AP1^T inhibited the adipocyte differentiation and modulated lipid metabolism-related genes, which may be caused by high level of propionate in cell-free supernatant.

Conclusions: Based on our results, It is expected that a novel propionate-producing bacterium AP1^T can be used as a next-generation probiotic for the treatment of metabolic disorders such as obesity, as propionate-producing consortium does.

Disclosure: No significant relationships.

Keywords: Korean gut microbiome, SCFA, obesity, Adipogenesis

SHIFT 02-196

Topic: ***AS28. Microbiomes and viromes***

NEORDRP: A COMPREHENSIVE DATASET FOR IDENTIFYING RNA-DEPENDENT RNA POLYMERASES OF VARIOUS RNA VIRUSES FROM METATRANSCRIPTOMIC DATA

Lecture Title:

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Background and Aims: RNA viruses are distributed throughout various environments, and most RNA viruses have been identified recently through metatranscriptome sequencing data analyses. However, owing to the high nucleotide diversity of RNA viruses, it is still challenging to identify novel RNA viruses from metatranscriptome data. To overcome this issue, we created a dataset named NeoRdRp containing hidden Markov models of RNA-dependent RNA polymerase (RdRp) domains that are essential for most of all RNA viruses.

Methods: 557,197 RdRp-containing amino acid sequences were collected as seed RdRp datasets. These sequences were processed through 1) deduplication (amino acid identity ≥ 99%) and clustering (amino acid identity ≥ 60%) using CD-HIT; 2) generating a multiple alignment for each cluster (≥ 3 sequences) using MAFFT; and 3) splitting a gappy region (25% gaps spanning eight or more alignment positions). Thereby, 19,394 HMM profiles were generated. We also evaluated the RdRp-containing sequences used for the NeoRdRp using InterProScan, RdRp-scan, and Palmscan.

Results: We evaluated the performance of NeoRdRp2 using the UniProtKB database containing 836 RdRp sequences out of 565,254 amino acid sequences. As a result, NeoRdRp2 hmmsearch (e-value ≤ 1E-10) detected 831 out of 836 RdRps in the UniProtKB database, while 188 were incorrectly identified as RdRp-containing sequences (i.e. recall and specificity rates were 99.4% and 81.6%, respectively). Comparisons of eight different RdRp (or RNA virus) search tools further showed that NeoRdRp2 exhibited balanced RdRp and nonspecific detection power.

Conclusions: The amino acid sequences and HMM profiles of NeoRdRp2 and their annotations are available at https://github.com/shoichisakaguchi/NeoRdRp.

Disclosure: No significant relationships.

Keywords: metatranscriptome, RNA virome, database, RNA-dependent RNA polymerase

SHIFT 02-197

Topic: ***AS31. Novel approaches to virus control***

ANTIVIRAL AND GENOTOXIC ACTIVITY OF WHITE-ROT FUNGAL SPECIES DAEDALEOPSIS CONFRAGOSA (BOLTON) J. SCHRÖT. 1888

Lecture Title:

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Background and Aims: *Daedaleopsis confragosa* a wood-rotting polypore fungus is known as the thin-walled maze polypore garnered attention due to its decay while its pharmacological importance has been overlooked, even though few studies have recently shown promising antimicrobial and antioxidative activity. In this study we investigated species from Serbia for its antiviral and genotoxic effects.

Methods: The phages were isolated from municipal waste water belonging to Caudovirales order with a genome made up of dsDNA molecules. Effect of DC extract (80 % EtOH, HD) on viral DNA integrity assay was done according to the following method - DNA was extracted from *P. aeruginosa* phages σ-2 (family Siphoviridae) and δ (family Podoviridae), previously purified via CsCl gradient by the phenol-chloroform extraction method. The impact of undiluted (1:1), 1:100, and 1:10000 diluted fungal extracts on the stability of viral DNA was evaluated after incubation for either 30 min or 24 h at 37°C, and the integrity of the DNA was assessed by analyzing agarose gel electrophoresis.

Results: After 30 min DNA σ-2 virus incubation with DC/DCHD extract indicated DNA disintegration although interpretation was hindered by the fluorescence that occurred due to the presence of UV-absorbing substances, probably phenolics determined (TPC 25.30, 12.12 mg GAE/g d.w., characterized by the presence of an aromatic ring with hydroxyl substituent or terpenoids reaching DPPH scavenging effect (IC_{50}8.53/10.13 μg/mL) detected by ESR. In addition 1:100 clearly disintegrate δ virus on gel.

Conclusions: Fungal biomolecules can directly affect the genotoxicity of viral nucleic acids and should be further investigated as possible novel antiviral agents.

Disclosure: Theauthorsgratefully acknowledge the financial support of the Ministry of Science,Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-66/2024-03/ 200125 & 451-03-65/2024-03/200125).

Keywords: fungi, phages, DNA fragmentation, Daedaleopsis confragosa, ESR

SHIFT 02-198

Topic: ***AS31. Novel approaches to virus control***

INNOVATIVE APPLICATION OF NBS SUPERFOOD AS AN ADJUVANT THERAPY TO ADDRESS THERAPEUTIC CHALLENGES IN INFECTIOUS DISEASES: CLINICAL TRIAL FINDINGS IN PATIENTS WITH COVID-19-RELATED ARDS

Lecture Title:

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Background and Aims: Addressing the challenges in treating infectious diseases, including pathogen resistance, drug inefficacy, and the emergence of new infectious agents, to develop more effective therapeutic strategies as adjunctive therapy.

Methods: The study randomly assigned 400 patients with Covid-19-related ARDS into two groups: intervention (n=200) and control (n=200). The intervention group received NBS powder (1.5 grams/day) for two weeks in addition to standard antiviral therapy, while the control group received a placebo with the same antiviral therapy. Blood samples were collected from all participants, and various laboratory parameters indicating inflammatory response and immune cell status were assessed.

Results: The intervention group displayed a statistically significant decrease in mean serum levels of inflammatory markers, including CRP (15.39 vs. 48.49, $p<0.001$), ESR (14.28 vs. 34.03, $p<0.001$), D-Dimer (485.18 vs. 1009.13, $p=0.001$), and CPK (68.93 vs. 131.48, $p<0.001$), highlighting the anti-inflammatory potential of NBS (Figure1). Additionally, a significant increase in mean lymphocyte count was observed in the intervention group (1537.06 vs. 1152.60, $p<0.001$) (Figure2). More patients in the intervention group normalized CRP, ESR, D-Dimer, and CPK levels compared to the control group ($p<0.001$). Additionally, the intervention group had a significantly lower mortality rate (8.5%) than the control group (31%, $p=0.001$), highlighting the potential life-saving impact of NBS supplementation alongside standard antiviral treatment.

Conclusions: NBS Superfood significantly reduced mortality rates in patients by modulating inflammatory responses and enhancing lymphocytic function. NBS Superfood has the potential to minimize the challenges of target-specific therapies and provide effective therapeutic assistance to patients regardless of the type of pathogens.

Disclosure: No significant relationships.

Keywords: complementary therapies, SARS-CoV-2 Omicron Variant

SHIFT 02-199

Topic: ***AS33. Other***

STUDY ON NOVEL SPECIES CANDIDATE OF GENUS PREVOTELLA ISOLATED FROM EQUINE RESPIRATORY INFECTIOUS DISEASE SPECIMENS

Lecture Title:

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Background and Aims: Properly isolating and identifying organisms from animal clinical specimens is crucial to health and antimicrobial resistance. This study characterized a novel *Prevotella* species isolated from equine clinical specimens.

Methods: Five strains of novel *Prevotella* species were isolated from equine respiratory tract disease specimens. All strains were isolated from different cases reported in various areas of Japan. Biochemical tests were performed using a Rapid ID 32A (BioMérieux). The full-length 16S rRNA gene and complete genome sequences of the strains were determined, and phylogenetic analyses were performed.

Results: All five strains formed non-pigmented colonies when cultured under anaerobic conditions at 37°C for 48 h on Brucella HK agar medium (RS) (Kyokuto Pharmaceutical Industries). Phylogenetically analyzing the full-length 16S rRNA gene sequences revealed that these strains formed an independent cluster among *Prevotella* species. The closest existing species sequences to this cluster were *Prevotella massiliensis* (AF487886) and *P. phocaeensis* (LN998069), with 94.94% and 91.2% homology, respectively. Phylogenetically analyzing the whole-genome sequences also indicated that these strains differed from existing species. These five strains showed almost identical and characteristic biochemical profiles to those of existing species.

Conclusions: These isolates belong to an independent taxonomic group and are novel bacterial *Prevotella* species. The isolated background suggests that this novel species is a possible pathogen that can cause equine respiratory infections.

Disclosure: No significant relationships.

Keywords: Taxonomy, Prevotella, Genome sequence, EQUINE

SHIFT 02-200

Topic: ***AS33. Other***

COMPARISON OF DIRECT STOOL ANTIGEN TEST AND INDIRECT SERUM ANTIBODY DETECTION METHODS FOR ACTIVE H. PYLORI INFECTIONS

Lecture Title:

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Background and Aims: Serology has been employed in large-scale surveys despite its reduced sensitivity and specificity, while stool antigen tests represent another non-invasive diagnostic method for identifying *Helicobacter pylori* infection. The aim of this study is to compare *Helicobacter pylori* antigen test (HpSA) and indirect serological method for detecting acute *Helicobacter pylori* infections.

Methods: A total of 106 patient sera for detection of anti- *H. pylori* antibodies and stool specimens for detection of *H. pylori* antigen were analyzed. The presence of clinical symptoms characteristic for *H. pylori* infection in patients was evaluated through a pre-validated questionnaire. The serological analysis was performed using enzyme-amplified chemiluminescent technology (IMMULITE®). Considering titers of anti-*H. pylori* IgG antibodies, samples were classified as seronegative (titer <1.1) and seropositive: low(titer 1.1-2), moderate (titer 2-3), and high (titer >3). Stool HpSA detection was performed using lateral flow assay method.

Results: HpSA positive results were obtained in 34 patients (32%). The agreement level between the two tests was found in 28/34 patients (82.8%), while inconsistent results were found in 6/34 patients (17.64%), who tested positive for HpSA but had negative serology. Of the seropositive samples 6/34 (17.64%), 10/34 (29.4%), and 12/34 (35.2%)samples had low, medium, and high titer, respectively. Thirty-three (97.05%) of patients positive for HpSA reported to have clinical symptoms characteristic of *H. pylori* infection.

Conclusions: The discrepancy between the results obtained from the direct antigen test and serology methods indicates that the use of only one of these two methods cannot be a reliable tool for the diagnosis of active *H. pylori* infection.

Disclosure: No significant relationships.

Keywords: Helicobacter pylori, serology, antigen

SHIFT 02-203

Topic: ***AS33. Other***

TO UNRAVEL THE ASSOCIATION OF HEPATITIS B VIRAL LOAD WITH CLINICAL LABORATORY PARAMETERS: A CROSS SECTIONAL STUDY IN A TERTIARY CARE HOSPITAL OF NORTHERN INDIA

Lecture Title:

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Background and Aims: Hepatitis B is the most prevalent virus that causes serious liver infection in the world. According to the current guidelines, HBV viral load along with other factors can help in treatment decisions. Therefore, the present study aims to explore the relationship between HBV viral load with blood-based laboratory parameters.

Methods: HBV Viral load was evaluated from blood samples of 159 HBsAg positive patients (ICT positive). Viral load was categorized into – high (above 200,000 IU/ml), moderate (between 2,000 to 200,000 IU/ml), and low (below 2,000 IU/ml). The viral load was then compared with clinical laboratory parameters.

Results: A significant association was observed between viral load and patient's age ($p<0.002$). Males exhibiting a substantially higher viral load (29.2%) compared to females (11%). A statistically significant association was found between viral load and AST ($p<0.005$), ALT ($p<0.04$) levels, with AST exhibiting pronounced alterations in low viral load cases, indicating liver dysfunction. A remarkable insight uncovered in our study revolves around the notable increase in serum calcium levels.

Conclusions: AST, ALT, and serum calcium levels were the most altered parameters with high HBV viral load. Though limited reports were available on the altered serum calcium levels, it could serve as a potential laboratory marker for assessing the disease progression in HBV infection. Moreover, focusing on potential therapies to normalize AST, ALT, and serum calcium levels could offer promising avenues for combating HBV infection.

Disclosure: No significant relationships.

Keyword: Hepatitis B Virus, Laboratory parameters, viral load, q-PCR, blood borne infection

SHIFT 02-204

Topic: ***AS34. Pathogenesis of viral diseases***

EFFECT OF TRYPTOPHAN TO TYROSINE MUTATION AT POSITION 61 ON SFTS VIRUS NPS AFFECTS VIRAL REPLICATION THROUGH AUTOPHAGOSOME MODULATION

Lecture Title:

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Background and Aims: In our prior investigations, we elucidated the role of the Tryptophan 61st to Tyrosine substitution in the nonstructural protein (NSsW61Y) in diminishing the interaction between nonstructural protein (NSs) and nucleoprotein (NP), thereby impeding viral replication. In this study, we intended to understand the involvement of NSs in the replication via modulation of autophagosomes.

Methods: Initially, we examined the impact of NP expression levels, a marker for replication, upon infection of HeLa cells with Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV), under conditions with or without inhibition of NP binding. Western blot analysis revealed a reduction in NP levels under NSsW61Y-expressing conditions. Furthermore, the expression levels of canonical autophagosome markers, p62, and LC3, decreased in HeLa cells expressing NSsW61Y revealing the involvement of individual viral proteins on autophagy. Subsequent experiments confirmed that NSsW61Y perturbs autophagy flux, as evidenced by reduced levels of LC3B and p62 upon treatment with chloroquine, an inhibitor of autophagosome-lysosome fusion.

Results: Lysotracker staining demonstrated a decrease in lysotracker-positive lysosomes in cells expressing the NSs mutant compared to those expressing wild-type NSs. We further explored the mTOR-associated regulatory pathway, a key regulator affected by NS mutant expression. The observed inhibition of replication may be linked to conformational changes in NSs, impairing its binding to NP and altering mTOR regulation, a crucial upstream signaling component in autophagy.

Conclusions: These findings illuminate the intricate interplay between NSsW61Y and the suppression of host autophagy machinery, crucial for the generation of autophagosomes facilitating viral replication.

Disclosure: No significant relationships.

Keywords: SFTSV, NSs, NP, replication, autophagosome

SHIFT 02-205

Topic: ***AS35. Phage bacteria interactions***

ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF PHAGES AGAINST PLANT PATHOGENIC BACTERIA PECTOBACTERIUM AND DICKEA, CAUSING SOFT ROT IN POTATOES

Lecture Title:

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Background and Aims: The fight against diseases of strategic agricultural crops (wheat, rice, soybeans, potatoes, etc) represents a big problem worldwide related to significant economic losses. Soft rot can be caused by microorganisms belonging to *Pectobacterium* and *Dickeya* genera of the Enterobacteriaceae family. The phage treatment can be considered as potential effective and safe tool for infection control in plants, including seed material. The aim of the study was to create a set of specific phages for subtyping of population of *Pectobacterium spp*. and *Dickeya spp*. isolates, also a combined phage preparation for detection and control of black leg and soft rot in potatoes.

Methods: Determination of lytic spectrum , lysis stability and Phage DNA restriction profiles was done according to standard methodology . The virion morphology of negatively contrasted samples was studied by TEM (Jeol 100SX).

Results: Twenty bacteriophages against *P. carotovorum* and *Dickeya spp*. were isolated and biologically characterized. Primary grouping of phages was done by phage plaque morphology and lytic spectrum against set of 60 strains of Pectobacterium and Dickeya . The phages demonstrated obvious host range diversity with cumulative coverage of more then 90% of strains.

Conclusions: Ten bacteriophages with overlapping spectrum were additionally characterized, including phage DNA restriction profiles, lysis stability in liquid culture and viability in various conditions. The mixture of finally selected 6 phages active to *Pectobacterium* and *Dickeya* was tested in preliminary challenge experiments - bioassays on potato discs under different regimenes. The inhibitory effect of phages on development of soft rot disease was shown.

Disclosure: No significant relationships.

Keywords: bacteriophage, plant pathogenic bacteria, biocontrol

SHIFT 02-206

Topic: ***AS35. Phage bacteria interactions***

GOSSIP PHAGES: LOOKING FOR COMMUNICATION IN THE MICROBIAL WORLD.

Lecture Title:

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Background and Aims: Arbitrium system is a quorum sensing mechanism that modulates the lysis-lysogeny decision in phages infecting Bacillota. First described in SPbeta phages infecting *Bacillus* species, arbitrium system is based on a small peptide (AimP) sensed by a receptor (AimR) which modulates the expression of a lytic gene or module. There are multiple arbitrium system encoded not only in phages but also in genomes host, plasmids, and other mobile genetic elements (MGEs) cluster into 10 different clades. Mature AimPs (6 to 12 amino acids) from each clade share high homology and some just differ in 1 or 2 amino acids. At first sight it was proposed that this communication mechanism only had vertical direction, between the phage and its progeny, but the abundance of arbitrium systems and its homology could lead to crosstalk between related arbitrium system from different MGEs, opening a new paradigm for arbitrium system.

Methods: In this work we have studied the possibility of crosstalk between different arbitrium system, by deepen in the AimR receptor capability of sense different peptides, using protein to protein interaction techniques, reporter strains and infection assays.

Results: We have demonstrated that AimR is able to recognize peptides with different intensity from distinct phages.

Conclusions: Our recent results, which will be presented in the communication, could lead to a new consideration of arbitrium system and imply that also could be a communication mechanism between MGEs to promote complex social behaviors, including competence, cheating, eavesdropping or collaboration.

Disclosure: No significant relationships.

Keywords: quorum sensing, arbitrium system, microbial communication, Bacillus

SHIFT 02-207

Topic: ***AS35. Phage bacteria interactions***

EXPLORING INDUCIBILE CAUDOVIRICETES PROPHAGES IN GRANA CHEESE PRODUCTION: CHARACTERIZATION AND IMPLICATIONS

Lecture Title:

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Background and Aims: Natural Whey Starter (NWS) are traditionally used in the production of cheeses like Trentingrana. This microbial ecosystem is carefully maintained through a back-sloping approach and comprised thermophilic lactic acid bacteria alongside bacteriophages. Deriving mainly form the raw milk, the presence of bacteriophage holds significant implications for cheese production technology, particularly concerning prophages. Thus, our study aims to characterize seven phages isolated from NWS used consistently over a year in Trentingrana cheese production.

Methods: Phages were characterized morphologically and genetically, with genomic data compared with existing information. Phenotypic analysis on the prophage lifestyle were conducted on their *Lactobacillus helveticus* hosts to identify induction conditions and assess their impact on acidification capability within an *in-vitro* NWS environment.

Results: Phages were classified within the *Caudoviricetes* class and assigned to the non-taxonomic phage types of myovirus and siphovirus. Harboring gene sets typical of phage cycle, these phages displayed numerous conserved ORFs, many with hypothetical/unknown functions and potentially linked to the specific “grana” ecological niche. The presence of putative integrase genes was confirmed phenotypically through prophage induction using Mitomycin C and other stressor condition within an *in-vitro* NWS setting.

Conclusions: This work offers new insights into the ecology of NWS phages, opening also new prospective in understanding the technological challenges in grana-like cheese production associated with phage activity.

Disclosure: No significant relationships.

Keywords: natural whey starter, hard cheese, Caudoviricetes, Lactobacillus helveticus, temperate phage

SHIFT 02-208

Topic: ***AS36. Plant-microbe interactions***

STRAW MULCHING EFFECTS ON SOIL FERTILITY AND MICROBIAL ACTIVITY IN RELATION TO POTATO GROWTH AND YIELD

Lecture Title:

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Background and Aims: Potato (*Solanum tuberosum*), a member of the Solanaceae family is the second most important crop in the Nordic countries. Northern Sweden is ideal for cultivating potatoes and the county Västerbotten used to be the centre of commercial potato production. However, due to the environmental change potato production has declined in northern regions of Sweden. Drought conditions at the time of plantation and heavy precipitation during the harvesting period affect the development and maturity of potatoes. To produce high quality yielding potatoes, selection is of high importance. Straw incorporation is a sustainable way to improve soil fertility thereby yield and stress tolerance in crops. Straw is rich in nutrients and indispensable organic amendments to bolster physicochemical and biotic soil properties. The present study examined the straw mulching effects on microbial diversity and finally its impact on yield, and quality parameters of potatoes (Mandel and King Edward cultivar).

Methods: The split plot design was utilized for the study. Further, the short-read amplicon sequencing was used to study the bacterial diversity in the samples collected from the soil exposed with and without straw. The 16S rRNA V3-V4 hypervariable region of bacteria is targeted for the study.

Results: The bacterial abundance is observed more in straw mulched soil than the non-mulched soil. The preliminary results suggested that straw mulching improved the quality of the potato cultivars (starch, ascorbic acid content and specific gravity of tubers).

Conclusions: Straw mulching improved the quality of the potato cultivars by structuring the bacterial communities and thereby soil fertility.

Disclosure: No significant relationships.

Keywords: Bacterial Diversity, Metabarcoding, Straw mulching, Potato quality, potato yield

SHIFT 02-209

Topic: ***AS38. Skin Neglected Tropical Diseases***

WHOLE-GENOME SEQUENCES OF THE YAWS BACTERIUM OF TANZANIAN NONHUMAN PRIMATE ORIGIN REVEAL EVIDENCE OF INTER-SPECIES TRANSMISSION

Lecture Title:

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Background and Aims: *Treponema pallidum* subspecies *pertenue* (*TPE*) is the causative agent of yaws, disease infecting humans in endemic regions in Africa, southern Asia and the Pacific region, and nonhuman primates (NHPs) in Africa. The infection is manifesting in form of papilloma or ulcers, and can progress into deformation of bone and cartilage if left untreated. Previously, the disease was believed to have no animal reservoir, which led to development of currently ongoing second yaws eradication campaign, known as Morges strategy, led by World Health Organization (WHO).

Methods: This work was focused on the *TPE* transmission in wild populations of NHPs, as they could serve as a possible source of reinfection of humans in the future, even when the disease is eliminated in humans. This work determined eleven whole *TPE* genomes from NHP isolates collected from three national parks in Tanzania: Lake Manyara National Park (NP), Serengeti NP, and Ruaha NP, from four species of NHPs: *Chlorocebus pygerythrus* (vervet monkey), *Cercopithecus mitis* (blue monkey), *Papio anubis* (olive baboon), and *Papio cynocephalus* (yellow baboon). Together with previously determined genomes of Tanzanian NHP *TPE* (n = 11), 22 whole-genome *TPE* sequences have now been analyzed.

Results: Comparing all genomes, five genome-to-genome comparisons revealed high degree of genetic similarity in samples collected from different NHP species.

Conclusions: This similarity is consistent with interspecies transmission of *TPE* among NHPs. *The work was funded by the National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103, Funded by the European Union - Next Generation EU) to DS.*

Disclosure: No significant relationships.

Keywords: yaws, Treponema, neglected tropical diseases, TPE, non-human primate

SHIFT 02-211

Topic: ***AS39. Virus discovery and virome studies***

STUDYING THE VIROME OF BATS IN KAZAKHSTAN

Lecture Title:

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Background and Aims: Bats have become an important monitoring object in virology in recent years. Bats are known as reservoirs of many viral infections dangerous to humans and other mammals. Screening of bats in southern Kazakhstan to study their local population as potential carriers of emerging viruses was conducted.

Methods: Sample collection was carried out by catching bats in hoop nets. Samples such as oral and rectal swabs, as well as blood serum, were obtained. Swabs were examined by massive parallel sequencing, and blood sera were examined in ELISA for antibodies to the influenza A virus.

Results: Studies of the bat virome have shown the presence of contigs of vertebrate viruses from the following genera and species: Adenovirus, Herpesvirus, Myotis ricketti retrovirus, Rousettus leschenaultii retrovirus. A study of the adenovirus genome showed that it belongs to a presumably new species of bat adenovirus of the Mastadenovirus genus. The genome of the bat herpesvirus turned out to be 94% similar to the Simplexvirus isolated from tropical bats.
In bats' virome, nucleotide sequences of the genomes of insect viruses of different families were also found: Grapevine leafroll-associated virus, Casphalia extranea densovirus, Dragonfly-associated circular virus, Danaus plexippus plexippus iteravirus, and Odonata-associated gemycircular virus-1. ELISA for antibodies to Influenza A viruses has shown negative results.

Conclusions: The identified bat viruses in Kazakhstan are highly diverse: they can be more than 90% similar to known viruses, or they can be novel species within known genera of bat viruses.

Disclosure: No significant relationships.

Keywords: Bat, Virome, Kazakhstan, Deep Sequencing, Myotis blythii

SHIFT 02-212

Topic: ***AS40. Virus dynamics in reservoir hosts***

INVESTIGATION OF APOBEC3 SENSITIVITY TO VIF-INDUCED DEGRADATION

Lecture Title:

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Background and Aims: Background: Feline immunodeficiency virus (FIV) and HIV belong to the Lentivirus genus of the Retroviridae, FIV shares structural, genetic, and transmission similarities with HIV, making it a valuable model for studying the pathogenesis of HIV-induced AIDS. The apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3 (APOBEC3) is one host restriction factor which can inhibit HIV and FIV by introducing hypermutations in viral genome. Conversely, HIV and FIV encode the viral infectivity factor (Vif) to degrade APOBEC3s in an ubiquitin-proteasome dependent pathway. Deciphering the intricate interplay between Vif and APOBEC3 holds promise for future HIV drug development. **Research aim**: To gain a more comprehensive understanding of the interplay between Vif and APOBEC3.

Methods: Methods: We conducted APOBEC3 degradation assays, luciferase reporter virus infection assays, Vif mutation assays, and Co-IP to investigate the interaction between APOBEC3 and Vif.

Results: Results: Feline A3Z3s inhibit ΔVif FIV, and related viruses from Pumas/Bobcats (PLVs). Feline Vifs can induce the degradation of feline A3Z3s by forming an E3 ligase complex. PLV1695 was experimentally introduced into a domestic cat to study lentivirus cross-species transfer. PLV1695 virus was controlled in the cat for unknown reason. We found that its Vif is unable to overcome the restriction imposed by feline A3Z3s. We identified a novel A3Z3 binding in Vif and explain why PLV1695 was restricted in cats.

Conclusions: Conclusion: The arms race between APOBEC3 and Vif is ongoing.

Disclosure: No significant relationships.

Keywords: APOBEC3, Vif, FIV, HIV