Lightening Talk Abstracts

Control Number: 2022-A-106-ASM-BIO

Topic 1: Host-associated Biofilms

Publishing Title: Pseudomonas aeruginosa Persister Cells Resist and Modulate the THP-1 Macrophage Immune Response

Author Block: **C. Hastings**, C. Marques;
Binghamton University, Vestal, NY.

Abstract Body: **Background:** Bacterial persister cells are a subpopulation of cells that have low metabolic activity and a physical tolerance to antimicrobials. These cells are present in both biofilm and planktonic populations. Pseudomonas aeruginosa, a clinically relevant pathogen, has been described as a model system for researching persister cells. While it is generally believed that persister cells evade the host immune system upon infection, the mechanisms and degree of evasion is poorly understood. In this work, we investigated whether several aspects of innate immunity affect persister cells similarly to the effects observed on the vegetative population. **Methods:** To achieve this, we made use of THP-1 macrophages as a model to study interactions of phagocytic cells with bacterial persister cells. We infected THP-1s with regular or persister cells, and quantified engulfment and elimination of internalized bacteria by viable counts. We also tested the bactericidal effects of human serum on persister cells. Finally, we measured secreted cytokine levels of IL-6, CXCL-8, IL-10, and TNF-α. **Results:** Engulfment of persisters was 2-Logs lower after 30 minutes of infection, and 1-Log lower from that point onwards up to 180 minutes of infection. Regular cells internalized at 90 minutes of infection were reduced by 1-Log following 24 hours, while no viability reduction was detected for persister cells. Human serum was able to eradicate regular cell populations by 24 hours, while persister cells were able to survive this treatment. By 90 minutes, macrophages infected with both populations secreted higher levels of IL-6 and TNF-α than control, although persister cells secreted significantly less than regular cells. Secretion of CXCL-8 was higher in THP-1s infected with regular cells, but statistically insignificant for persister infections compared to control. Secretion of IL-10 was higher than control in regular and persister infections, being significantly higher in persister infections after 90 minutes. **Conclusions:** Our research further reveals that persister cells evade engulfment, and that once engulfed, survive inside the phagosome. We discovered that persisters are bound with complement protein c3b with the same efficiency as regular populations, but persisters are resistant to complement-mediated killing. We also discovered that macrophages respond to persister cells, resulting in different levels of cytokine secretion relative to the vegetative population, which modulates the overall type of immune response occurring.

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Control Number: 2022-A-160-ASM-BIO

Topic 1: Natural Biofilms, Consortia and Simulations

Publishing Title: SHIP HULL COATINGS INFLUENCE THE STRUCTURE AND RHEOLOGICAL PROPERTIES OF MARINE BIOFILMS WITH IMPLICATIONS FOR DRAG

Author Block: **A. Jackson**[1], A. Finnie[2], S. Dennington[1], J. Longyear[2], J. Wharton[1], P. Stoodley[3]; [1]Southampton University, Southampton, UNITED KINGDOM, [2]AkzoNobel, Gateshead, UNITED KINGDOM, [3]Ohio State University, Columbus, OH.

Abstract Body: **Background:** Marine biofilms on ship hulls increase frictional drag. The physico-mechanical properties of these biofilms are hypothesised to be significant in influencing drag yet to our knowledge have not been rheologically characterised. Here we characterised the mechanical properties of marine biofilms and determined the effect of two types of commercial coatings on biofilm physico-mechanics. **Method:** Sanded circular PVC coupons (4 cm dia.) were coated with two commercially available coatings: a foul release coating (FRC), an anti-corrosive primer (ACP) or left uncoated (PVC). Coupons were deployed in an indoor recirculating seawater tank at the Dove Laboratory (Cullercoats, UK). Marine biofilms were cultivated for two-months at temperatures of 7 to 10^{o}C. Optical Coherence Tomography (OCT) was used to quantify structure of the biofilms - thickness, roughness, and percent coverage. A parallel plate rheometer was used to mechanically characterise the biofilms using amplitude sweeps, frequency sweeps and creep-recovery tests. Shear modulus (G), viscosity (η) and elastic relaxation time (λ) were quantified for all biofilms and qualitative microscopy was used to visualise the marine biofilm community. **Results:** Biofilms grown on FRC coupons were patchy and significantly thinner than those biofilms grown on ACP and PVC coupons ($P < 0.05$). Creep data revealed that FRC biofilms exhibited soft and viscous properties with a shear modulus (G) of 3.8×10^3 Pa and a viscosity (η) of 6.8×10^5 Pa s, whereas the ACP biofilms were stiffer and less viscous. In addition, the FRC biofilms possessed an elastic relaxation time (λ) more than three times that of the ACP and PVC biofilms. Despite differences in compliance, all biofilms tested were brown which suggests a heavy diatom presence. **Conclusion:** Marine biofilms are viscoelastic, and surface coating alters their physico-mechanical properties. By determining how the mechanical properties of biofilms are altered by fouling control coatings, and understanding how these properties interact to implicate drag, then future fouling control coatings could be designed to show increased efficiency in controlling specified biofilm properties.

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Control Number: 2022-A-202-ASM-BIO

Topic 1: Synthesis, Assembly and Function of Extracellular Biofilm Determinants

Publishing Title: LIPOPROTEIN MUTATIONS THAT IMPACT BIOFILM MORPHOLOGY IN UROPATHOGENIC *ESCHERICHIA COLI*

Author Block: **H. D. Green**, T. Williams, G. Van Horn, M. Hadjifrangiskou, J. Schmitz; Vanderbilt University, Nashville, TN.

Abstract Body: **Background:**Uropathogenic *Escherichia coli* (UPEC), the primary cause of urinary tract infections (UTIs), forms biofilms on catheter devices, on the urothelial surface

and inside host cells. Two primary biofilm matrix components in UPEC are cellulose and curli amyloid fibers, both of which take up the Congo Red (CR) dye when bacteria are spotted on CR-supplemented agar plates. In this work, we identified clinical UPEC isolates that form colony biofilms with prominent intra-strain heterogeneity in their ability to take up CR. We focused on a previously poorly characterized morphotype that forms a *"peppermint"* pattern, because isolates with this "peppermint" morphotype were more frequently isolated from patients with recurrent UTIs (rUTIs). Here, we uncover genetic mutations associated with the peppermint phenotype of one peppermint isolate deriving from rUTIs, VUTI148.**Methods:** Bacterial strains were grown overnight in Luria broth and 10ml of overnight culture was spotted on CR-supplemented agar plates. Colony biofilms were incubated for 11 days at room temperature and images were taken of biofilm development at 11 days. Red and white subpopulations from the colony biofilm were isolated and subjected to gDNA isolation and next generation sequencing on an Illumina platform. Obtained reads were compared between white and red samples. Deletion of identified genes was performed using lambda-red recombination.**Results:** Genomic analyses of red subpopulations uncovered 4 independent mutations in the *nlpI* gene that encodes an outer membrane lipoprotein. A clean *ΔnlpI* mutant phenocopied the *nlpI* mutant subpopulations, displaying increased rugosity and higher CR uptake. Increased rugosity is not due to differences in cellular replication, because colony biofilms from Δ*nlpI* and the wild-type strain have similar bacterial titers.

Conclusion:

We postulate that loss of NlpI leads to changes in the extracellular matrix of UPEC, possibly through the regulation of curli and cellulose.

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Control Number: 2022-A-205-ASM-BIO

Topic 1: Innovative Approaches and New Technologies in Biofilm Research

Publishing Title: ELECTROCHEMICAL QUANTIFICATION OF *P. AERUGINOSA* BIOFILMS, AND REDUCTION USING EXTRACTED BACTERIAL SECONDARY METABOLITES

Author Block: **L. Riordan**, P. Lasserre, D. Corrigan, K. R. Duncan;
University of Strathclyde, Glasgow, UNITED KINGDOM.

Abstract Body: Background *Pseudomonas aeruginosa* has shown resistance to nearly all classes of antibiotics, and is routinely responsible for implant infections, with these biofilms often undetected until the patient develops bacteraemia or the implant is explanted. Therefore, new technologies to detect biofilms earlier are needed, as well as new antibiofilm-metabolites to inhibit formation and target established biofilms. Such techniques as electrochemical impedance spectroscopy (EIS) and square wave voltammetry (SWV) which measure biofilm formation on an electrode and detect redox-active secondary metabolites in the growth media above the electrode, respectively. Methods In this study, we highlight the application of EIS and SWV in a 96-well plate format on carbon electrodes. These methods can be applied to real-

time monitoring of biofilm formation and were validated using crystal violet (CV) staining of *P. aeruginosa* (PA14 & LESB58) biofilms at a concentration range (OD600 0.05-1). We have also isolated 11 actinomycetes from Scottish marine sediments and extracted their metabolites from liquid culture. These metabolite extracts have been quantified for their ability to prevent *P. aeruginosa* biofilm formation using EIS, SWV and CV. Results An increased current amplitude (μA) in SWV measurements indicates planktonic cell growth, and a decrease in impedance (Z) indicates biofilm formation. SWV peak amplitudes at -0.35 V at 0, 2 and 4 hours, gave 6.47, 18.27 and 29.61 μA respectively for OD600 1 LESB58 biofilms, and 0.63, 3.97 and 9.64 μA for PA14 biofilms at the same time points and starting concentration, and directly correlated to pyocyanin production. Whilst those exposed to 1 mg/mL of metabolite extract showed no significant change. Over 4 hours, impedance (Z) decreased from 3680 to 1797, and 3775 to 849 for PA14 and LESB58 biofilms respectively, and this can likely be attributed to *P. aeruginosa* biofilm formation, as there was no significant difference in EIS measurements over the 4 hours for the media only control (3782 at 0 hours, and 3378 at 4 hours). Conclusions Our results show that we have successfully extracted an antibiofilm agent which is active against *P. aeruginosa* biofilm formation, and this can be quantified using electrochemical approaches. Next, *P. aeruginosa* biofilms will be formed on medically relevant materials and the ability of the metabolite extracts to reduce these biofilms will be quantified using EIS and SWV.

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Control Number: 2022-A-218-ASM-BIO

Topic 1: Control, Prevention and Elimination of Biofilms

Publishing Title: Development of clinically relevant *in vitro* and *in vivo* intravenous catheter biofilm infection models for studying the efficacy of lock therapy

Author Block: **Y. Yoshii**, S. Thiriet-rupert, A. Chauhan, D. Lebeaux, J. Ghigo, C. Beloin; Institut Pasteur, Paris, FRANCE.

Abstract Body: **Background:** Central venous catheters (CVCs), including totally implantable venous access ports (TIVAPs), are medical devices widely used for the long-term administration of antineoplastic drugs or parenteral nutrition. The use of CVCs involves a risk of microbial contamination leading to biofilm formation on/in the device and subsequent critical biofilm infections. However, for some patients, TIVAPs are a life-saving technique; it is challenging to remove the device, even though such a catheter biofilm infection is caused. Thus, antibiotic lock therapy (ALT) is performed to salvage the infected device. Usually, 100-1,000 times MIC of antibiotics is continuously (or sometimes intermittently) injected for 7-14 days into the catheter and exchanged every 24-72 hours. However, the effects of ALT are not fully established since the infection sometimes relapses by resistant or tolerant bacteria. Although some fundamental research models are proposed to investigate the efficacy of ALTs, those models lack clinical relevance since small pieces of an infected catheter with bacteria are inoculated into a closed culture system or an

animal's subcutaneous space in the models. Hence, developing clinically relevant *in vitro* and *in vivo* TIVAP biofilm infection models can be important for studying the efficacy of ALT (and risks of antibiotic-resistant bacterial emergence). **Method:** 1) *In vitro* model; by combining a continuous flow system and a pediatric TIVAP, we developed an *in vitro* TIVAP model to mimic catheter-related infections. 2) *In vivo* model; by implanting a catheter part into the rat's jugular vein and a port part into the back, we established an *in vivo* TIVAP model. **Results:** 1) The *in vitro* model allowed *E. coli* LF82 to form biofilms on the TIVAP under continuous flow conditions. Ten-day, but not 7-day, amikacin lock therapy at 500-fold MIC eradicated biofilm cells on the TIVAP without resistance emergence. 2) The *in vivo* model allowed *in situ* monitoring of bioluminescent *E. coli*, *S. aureus, and P. aeruginosa* biofilms in living rats. Similar to 1), the *in vivo* model showed decreased bioluminescent intensities of biofilm cells after ALT. **Conclusions:** Our *in vitro* and *in vivo* models can be applied to studying the efficacy of ALT and the relationship of intermittent ALT with resistant bacterial emergence. Studies combining these two models will allow the evaluation of new strategies against catheter biofilm infections on the path toward clinical application.

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Control Number: 2022-A-222-ASM-BIO

Topic 1: Metabolism, Physiology and Structure of Biofilms

Publishing Title: THE ROLE OF EXTRACELLULAR DNA IN MUCOID *PSEUDOMONASAERUGINOSA*BIOFILMS

Author Block: **D. Ferguson**[1], E. S. Gloag[2], D. J. Wozniak[1];
[1]The Ohio State University, Columbus, OH, [2]Virginia Tech, Blacksburg, VA.

Abstract Body: **Background** *Pseudomonas aeruginosa* (PA) is one of the most common biofilm-forming pathogens responsible for lung infections of immunocompromised individuals, such as people with cystic fibrosis (CF). Mucoid variants of PA are frequently isolated from CF sputum samples and are associated with worsening patient outcomes. Mucoid variants overproduce the exopolysaccharide, alginate, that impedes antimicrobials and host defenses. This study investigates how other biofilm matrix polymers, such as extracellular DNA (eDNA), contribute to mucoid biofilm structure and physical properties. eDNA is a major structural component for nonmucoid biofilms, but its role in clinical mucoid biofilms remains unclear. **Methods** Biofilm eDNA was isolated and quantified to elucidate the abundance in mucoid biofilms, compared to nonmucoid. Mucoid biofilms were fluorescently labelled and visualized by microscopy to quantify eDNA at different timepoints during biofilm development. Uniaxial indentation and squeeze pull-off assays were performed to detect changes in biofilm biophysical properties, with and without DNase I treatment. **Results** Our studies show that eDNA levels in PA mucoid biofilms is generally lower than those formed by nonmucoid strains, although biofilm eDNA abundance is diverse across several clinical mucoid strains. Images of mucoid biofilms show an increase in eDNA production over the course of development. Biophysical analyses reveal that DNase I treatment significantly

increases biofilm stiffness but decreases biofilm thickness and cohesion of those mucoid biofilms with high eDNA abundance. This effect was negligible on biofilms with low eDNA production. Moreover, clinical mucoid isolates with low eDNA abundance formed biofilms that were stiffer and thinner when compared to those with high eDNA. **Conclusions** Degradation of eDNA impacts the biophysical properties and integrity of clinical mucoid biofilms that produce high levels of eDNA. The diversity in abundance of eDNA amongst the various clinical isolates and susceptibility to DNase I aligns with the complexity of treating adapted PA variants in the biofilm state. These findings can lead to new approaches for targeting biofilms by exploiting the functions of matrix components to increase effectiveness of therapeutics in infected patients.

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Control Number: 2022-A-277-ASM-BIO

Topic 1: Signals and Mechanisms Promoting Biofilm Disassembly and Dispersal

Publishing Title: INVESTIGATING *SALMONELLA ENTERICA* SEROVAR *TYPHIMURIUM* BET-HEDGING USING A DUAL REPORTER STRAIN

Author Block: **M. I. Gerber**, D. J. Herman, M. B. Palmer, C. R. Nnajide, M. C. McCarthy, J. Sparrow, N. Dhar, A. P. White;
Vaccine & Infectious Disease Organization-International Vaccine Centre, Saskatoon, SK, CANADA.

Abstract Body: **Background** Non-typhoidal *Salmonella* are one of the leading causes of food-borne illness worldwide (1). These bacteria cycle between living in hosts and persisting in the environment in biofilms (2). When grown in biofilm conditions, *Salmonella enterica* serovar *Typhimurium* 14028 (*S. Typhimurium*) differentiates into biofilm aggregates and virulent single cells (3). We think this population split is a bet-hedging strategy to increase transmission, providing a way to cause disease immediately (single cells) or after long time periods (aggregates). To study bet-hedging in more detail, we built a fluorescent reporter strain of *S. Typhimurium* that tracks biofilm formation (GFP) and virulent type III secretion synthesis (mCherry) simultaneously. Our goal is to characterize the genetic program that leads to biofilm cells and SPI-1 T3SS^{+} single cells forming within the same population. **Methods** We built the dual fluorescent construct using Golden Gate Assembly, moved it into the *S. Typhimurium* genome by Tn7 transposition (4), and confirmed the sequence with Nanopore technology. We grew the dual reporter strain in an *in vitro* flask model for biofilm development (3). Biofilm aggregates and single cells were separated, homogenized, and examined on microscope slides. Approximately 5000 cells were counted per slide and the mean GFP and mCherry signals quantified using ImageJ. **Results** We identified four different cell types and characterized their proportions within each sample. The total population consisted of ~50% biofilm cells, with SPI-1 T3SS^{+} single cells and non-fluorescent cells making up the other half (Table 1). There was also a rare population of double positive cells in biofilm and single cell samples. We have started to study the *S. Typhimurium* cell populations *in vivo* in a *Caenorhabditis elegans* infection model. **Conclusions** We

identified four distinct cell types that form during *S. Typhimurium* bet-hedging. There could be at least two bistable regulatory networks: biofilm^{+} and biofilm^{-} (5) and fluorescent SPI-1^{+} cells and NF cells, which we assume represent a SPI-1 OFF population (6). In the *C. elegans* infection model, we will image worms at 8-24 hours post infection to determine if the same populations exist *in vivo*.

Proportion of S. Typhimurium Cell Types Formed in a Biofilm Flask Model

Cell Type	**Biofilm Samples**	**Single Cell Samples**	**Total Population**
Biofilm+ GFP+/mCherry-	0.88	0.08	0.49
SPI-1 T3SS+ GFP-/mCherry+	0.01	0.51	0.26
Biofilm+ & SPI-1 T3SS+ GFP+/mCherry+	0.03	0.02	0.02
Biofilm-/SPI-1 T3SS- GFP-/mCherry-	0.08	0.39	0.23

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Control Number: 2022-A-303-ASM-BIO

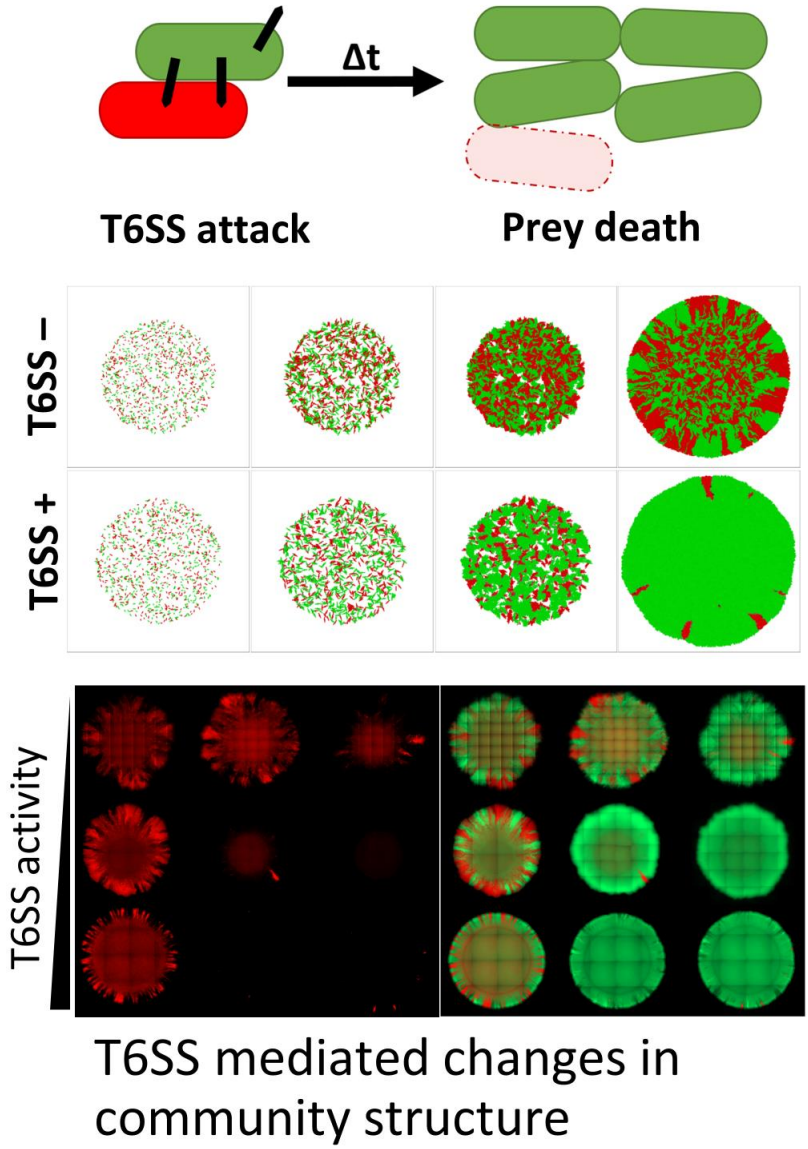
Topic 1: Social and Asocial Interactions in Biofilms

Publishing Title: How *Pseudomonas aeruginosa* uses Type VI Secretion system to shape the structure of polymicrobial communities

Author Block: **M. Rudzite**, A. Filloux;
Imperial College London, London, UNITED KINGDOM.

Abstract Body: **Background** *Pseudomonas aeruginosa* is an opportunistic pathogen and its biofilm lifestyle is associated with chronic infections. To establish and persist within dense polymicrobial biofilm communities *P. aeruginosa* utilises Type VI Secretion systems (T6SS) for direct competition. T6SS is a macromolecular weapon used to inject toxins into adjacent prey cells. *P. aeruginosa* possesses 3 distinct T6SSs and a set of more than 20 toxic effectors. T6SS toxins poses a diverse set of functions including disruption of cell wall integrity, degradation of nucleic acids, or metabolic impairment. **Methods** We generated a collection of mutants with various degrees of T6SS activity and/or sensitivity to individual T6SS toxins. By imaging whole mixed bacterial macrocolonies, we aimed to investigate how these *P. aeruginosa* strains gain a competitive edge in polymicrobial communities. The experimental work is supported by agent-based theoretical simulations aimed at pinpointing how small mechanistic differences in T6SS attacks affect whole community structure. **Results** Disruption of Gac/Rsm cascade leads to a gradual elevation in T6SS activity. Using mutant strains with varied T6SS activity levels we demonstrated that bacterial community structure can be affected by the action of every single T6SS toxin and how toxin synergy maximises impact on prey growth.

Additionally, we demonstrate how the establishment of direct prey-attacker contact is a key component for successful T6SS-based competition. **Conclusions** This study of T6SS-dependent competition in whole bacterial colonies addresses how toxin multiplicity and other factors like changes in T6SS firing behaviour or cell-cell contacts lead to population-level competitive advantages. Overall furthering the understanding of how highly local contact-based interactions between individuals shape the structure of whole populations, thus providing conceptual insights applicable to all types of contact-based competition.



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Control Number: 2022-A-312-ASM-BIO

Topic 1: Control, Prevention and Elimination of Biofilms

Publishing Title: FIBRIN BASED NANOPARTICLE INFILTRATION OF STAPHYLOCOCCUS AUREUS BIOFILMS

Author Block: **G. Scull**[1], A. Aligwekwe[1], D. Koch[2], J. Sollinger[2], A. Sheridan[1], J. Gilbertie[3], L. Schnabel[2], K. Nellenbach[1], A. C. Brown[1];
[1]North Carolina State University & UNC-Chapel Hill, Raleigh, NC, [2]North Carolina State University, Raleigh, NC, [3]Edward Via College of Osteopathic Medicine, Blacksburg, VA.

Abstract Body: **Background:** *S. aureus* is a gram-positive bacteria implicated in diseases such as endocarditis and chronic dermal wound infection. *S. aureus* generates fibrin-based biofilms by hijacking host wound healing machinery via Clumping factor A (ClfA) and coagulase, enhancing immune evasion and antimicrobial recalcitrance. The Brown Lab has developed Fibrin Based Nanoparticles (FBNs) that can be loaded with therapeutics and delivered to the infection site for *S. aureus* biofilm incorporation and targeted drug release. **Methods:** FBNs were synthesized via fibrinogen enzymatic conversion into fibrin and sonication into nanoparticles. Particles were characterized using an Asylum atomic force microscope (AFM) and Malvern NanoSight nanoparticle tracking analysis (NTA) (1A). FBNs were loaded with vancomycin (vFBN) or vancomycin+tissue plasminogen activator (tPA) (dFBN), and release characterized via plate reader and ELISA (1B). FBN interaction with *S. aureus* and incorporation into fibrin biofilms was identified via confocal microscopy (1C). Loaded FBN kill potential was analyzed via bacterial CFU assay *in vitro* (1D) and murine dermal wound model *in vivo* (1E). **Results:** FBNs had a dry diameter of 213 ± 17 nm, hydrodynamic diameter of 295 ± 57 nm, and dry height of 15 ± 5 nm. Results show vancomycin loading of 0.29 pg for vFBN (38% efficiency) and 0.24 pg for dFBN (35% efficiency) per FBN particle, and 0.007 pg tPA per FBN particle (30% efficiency). *S. aureus* interacts with FBNs as it does native fibrin and incorporates FBNs throughout the biofilm full thickness. vFBNs reached clinically significant 3 log(CFU/ml) killing efficiency *in vitro*, and trends suggest a decrease in Xen36 radiance and burden *in vivo*. **Conclusions:** FBNs provide a novel, biocompatible approach for the administration of antimicrobial therapeutics to fibrin-

based bacterial biofilms.

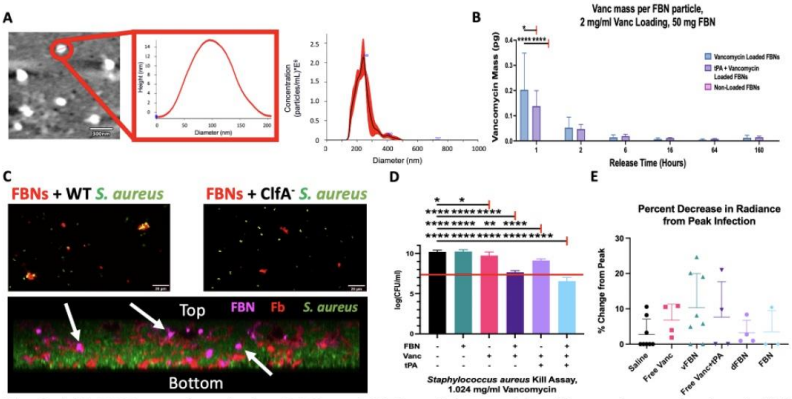

Fig 1. (A) FBNs analyzed via AFM and NTA, with particle dimensions obtained. (B) vFBNs show initial burst release profile over time. (C) Alexafluor 647-FBNs were combined with baclight green-stained ClfA$^{+/-}$ USA300, with yellow regions indicating binding of bacteria to FBNs. 647-FBNs were added to a biofilm of GFP *S. aureus* and 546-fibrinogen, and confocal cross-section shows FBNs are incorporated throughout the biofilm. (D) Clinical strain AK was used in a kill assay, with red bar showing significant 3 log(CFU/ml) decrease in bacterial concentration. (E) Radiance signal from IVIS imaging of mouse dermal wounds infected with bioluminescent Xen36 suggest there may be a positive *in vivo* response to vFBN treatment.

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Control Number:	2022-A-327-ASM-BIO
Topic 1:	Social and Asocial Interactions in Biofilms
Publishing Title:	FORMATION AND BEHAVIOR OF ZORBS—MOTILE, MULTI-SPECIES BACTERIAL BIOFILMS
Author Block:	**S. Magesh**, J. F. Nepper, C. Li, D. J. Beebe, J. Handelsman; University of Wisconsin-Madison, Madison, WI.
Abstract Body:	**Background:** Most, if not all, microorganisms form multicellular communities known as biofilms in natural environments. Most bacteria form static biofilms in which cells adhere to a surface, multiply and form micro-colonies that eventually mature and produce extracellular polysaccharides. However, *Flavobacterium johnsoniae* forms motile, biofilm-like micro-colonies called zorbs. **Methods:** We observed zorb formation in an under-oil microfluidic system using time-lapse microscopy for 16 hours. **Result:** We previously reported that zorbs move using cells at the base of the structure that are attached to the surface by one pole of the cell. Here, we report that several mutants defective in surface colonization failed to form zorbs whereas mutants with enhanced surface colonization capabilities produced larger, faster-moving zorbs, suggesting that surface colonization is important for zorb formation. Because microorganisms exist in communities, we tested the ability of *F. johnsoniae* to form zorbs with other bacteria. *F. johnsoniae* has been previously identified as a biological hitchhiker of an ecological neighbor, *Bacillus cereus*. When co-inoculated with *F. johnsoniae*, *B. cereus* occupied the core of *F. johnsoniae* zorbs, a phenomenon we call “co-zorbing.” **Conclusion:** This work demonstrates a novel interspecies interaction and

demonstrates the utility of the under-oil system in the study of multi-species biofilm formation.

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Control Number: 2022-A-343-ASM-BIO

Topic 1: Innovative Approaches and New Technologies in Biofilm Research

Publishing Title: IDENTIFYING THE GENETIC DETERMINANTS OF G.*VAGINALIS* BIOFILM FORMATION

Author Block: **J. Magri**, M. Topf, J. Kernien, N. M. Nightingale, K. A. Overmyer, J. J. Coon, C. S. Pepperell;
University of Wisconsin-Madison, Madison, WI.

Abstract Body:
Background: *Gardnerella vaginalis* (GV) is an integral member of the vaginal microbiome and is considered to be the main causative agent of the common reproductive disease bacterial vaginosis (BV). BV pathogenesis is spurred by "dysbiosis" of the vaginal microbiome, characterized by GV overgrowth in a multispecies biofilm (Machado & Cerca, 2015). GV has been hypothesized to act as the foundation of the BV biofilm; however, little is known about GV's role in biofilm formation and maintenance. Because BV is a risk factor for preterm birth, acquisition of STIs, and pelvic inflammatory disease (Hay, 2017), it represents a substantial global threat to health. Therefore, determining GV's role in biofilm formation could reveal new targets for antibiotics and new treatments for BV.
Methods: To identify the genetic determinants of biofilm formation in *G. vaginalis*, I created an *in vitro* system that uses experimental evolution to select for biofilm cells. Nine GV cultures (GV2 strain) were evolved independently with serial passaging, and optical density and crystal violet stain measurements for each biofilm were recorded over time to determine phenotypic change. Whole genome sequencing of ancestral and evolved biofilm populations identified significant changes in allele frequencies.
Results: Experimental evolution of GV biofilm populations resulted in a general increase in biofilm density over time. Sequencing data also showed that across nine iterations of evolution, the same gene (GAVG_1022) was hit multiple times in our evolved strains; GAVG_1022 was later found to code for a type 1 fatty acid synthase (FAS). All mutations in GAVG_1022 were nonsynonymous, and five of the seventeen total mutations occurring in this gene occurred in the keto-reductase (KR) domain, with others hitting various protein-coding domains. Subsequent lipidomics revealed an increase in production of certain lipids in three evolved strains compared to the ancestor, suggesting that the mutations may affect the rate at which those lipids are produced rather than altering fatty acid profiles.
Conclusions: Our data indicate that pressure to grow as a biofilm spurs an increase in GV biofilm production over time, and experimental evolution of GV biofilms has pinpointed the type 1 FAS locus as a locus operating under positive selection for biofilm growth. Future directions will focus on elucidating the role of different fatty acids in biofilm production and the impact of specific type 1 FAS mutations on biofilm formation.

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Control Number:	2022-A-379-ASM-BIO
Topic 1:	Susceptibility and Tolerance: Antimicrobials and Biofilms
Publishing Title:	PHOENIX COLONY EMERGENCE in *PSEUDOMONAS AERUGINOSA* BIOFILMS
Author Block:	**D. Sindeldecker**, P. Stoodley; Ohio State University, Columbus, OH.
Abstract Body:	**Background:** *Pseudomonas aeruginosa* is an important biofilm-forming, opportunistic, bacterial pathogen that causes approximately 51,000 nosocomial infections per year including cystic fibrosis-related infections, post-surgical infections, and wound infections. Variant like antibiotic tolerant or resistant colony generation has previously been shown in biofilm cultures exposed to tobramycin which could lead to recurrent or persistent infections. **Methods:** In this study, variant colonies were generated by exposing lawn biofilms of *P. aeruginosa* to tobramycin. After generation, colonies were isolated and plated for minimum inhibitory concentration (MIC) testing. Growth curves were also generated for the isolates to look for the presence of a growth defect that could explain their survival. In addition to variant colony characterization, the antibiotic concentration throughout the agar over time was also characterized using a modified Kirby-Bauer style protocol. After variant colony characterization, RNAseq was performed on the variant colonies. **Results:** Three phenotypes were identified from the variant colonies which survived the tobramycin exposure - classically resistant colonies, and two novel variants, viable but non culturable colonies (VBNC)-like colonies, and a tolerant phenotype which we call “phoenix” colonies. Phoenix colonies are variants which grow and remain active within the zone of clearance of tobramycin, while there are still high levels of antibiotic present, but upon subculturing, return to wild-type susceptibility. They show no growth defects indicating they are not persister cells. RNAseq of phoenix colonies identified genes which may play a role in phoenix colony survival, including a tRNA pseudouridine synthase, which may interact with the aminoglycoside leading to antibiotic tolerance. **Conclusions:** These findings suggest that resistant colonies, VBNCs, and phoenix colonies may be generated in biofilms exposed to antibiotic therapies, but by achieving high local antibiotic concentrations, these variant colonies may be able to be killed reducing the incidence of recurrent or persistent infection. Additionally, novel phoenix colony antibiotic tolerance may be conferred through an interaction between tRNA pseudouridine synthases and aminoglycosides.

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