A133
CHARACTERIZATION OF BIOFILMS FORMED BY ANAEROBIC GASTROINTESTINAL BACTERIA


Background: An imbalance of intestinal microflora or association of specific pathogens with the intestinal mucosa may contribute to the development of gastrointestinal diseases such as Inflammatory Bowel Disease. Studies of intestinal microflora have generally focused on the characterization of microorganisms in free-swimming form. Recently, studies have demonstrated that microbes grow as biofilms on the gastrointestinal mucosa. The study of biofilms is therefore an important step in understanding the complex nature of the intestinal microflora. This study aimed to characterize the community structure of biofilms formed by mucosal bacteria isolated from the human colon.

Methods: Mucosal biopsies of the colon were obtained from healthy patients undergoing screening colonoscopy. Homogenates of the biopsies were used to seed anaerobic biofilms in the Calgary Biofilm Device. Biofilm growth was assessed by viable cell counting and confocal scanning laser microscopy (CSLM), and biofilm community structure was assessed using polymerase chain reaction (PCR), quantitative PCR, and terminal restriction fragment length polymorphism (T-RFLP).

Results: The anaerobic biofilms developed quickly and continued to increase in size over a period of up to five days. CSLM imaging of the biofilms demonstrated dense, three-dimensional structures, with biofilms reaching a thickness of between 20 and 80 µm after five days of growth. The biofilm communities showed great diversity, with PCR analysis detecting bacteria from the Bacteroides-Prevotella group, Clostridium coccoide group, Enterobacteriaceae, and the following genera: Bacteroides, Clostridium, Desulfovibrio, Escherichia, Faecalibacterium, Lactobacillus and Ruminococcus. Community composition, as measured by quantitative PCR, shifted during the growth period.

Conclusion: Complex biofilm communities were formed using mucosal bacteria from the human colon. These biofilm communities serve as a "representative microflora", which will be useful in further characterization of host-microbe interactions in the gastrointestinal tract.

Acknowledgments: Biopsies were provided by the Intestinal Inflammation Tissue Bank at the University of Calgary. This work was supported by CCFC, NSERC and AHFMR.

B134
CSPA PARALOGUES AFFECT S. TYPHIMURIUM PERSISTER FORMATION

S. E. Spragg, I. W. Hutchinson, W. C. Poon, M. P. Gallagher; University of Edinburgh, Edinburgh, UNITED KINGDOM

Background: Persister cells survive antibiotic treatment due to their temporary dormant state and endure in biofilms. We have constructed a (null) mutant of Salmonella enterica sv. Typhimurium, lacking all cold shock protein (cspa) paralogues required for growth after cold shock to 10°C. At 10°C, this strain is unable to divide, remains viable and persists for several weeks but is capable of regrowth at 37°C. Thus, Null cells are conditionally dormant. Using this Null mutant we aim to investigate persister formation in planktonic cultures and develop an assay to identify individual persisters within biofilms.

Methods: Stationary phase cultures of S. Typhimurium SL1344 (WT) and Null were compared at 37°C or after 2 hours of cold shock and subsequent incubation at 10°C in LB. Ofloxacin sensitivity (5µg/ml) was explored +/- chloramphenicol pre-treatment (20µg/ml, 30min) and viability determined over time. MIC values were determined using a broth dilution method.

Biofilms were imaged in Nunc® Chambered Coverglasses using confocal microscopy and plasmid-encoded GFP. Results: MICs of the WT & Null strain for ofloxacin were found to be identical at 37°C (0.125µg/ml). However treatment of stationary phase cultures with 5µg/ml revealed hypersensitivity of the Null (vs. WT) at both 37 & 10°C, which could be prevented by cspE expression, implicating a role for cspA paralogues in persister formation. Chloramphenicol also reduced hypersensitivity at 37°C, linking translation with persister formation.

Surprisingly cold shock (10°C) substantially increased persister levels, although Null cells remain hypersensitive. Imaged biofilms showed no observable differences between WT & Null at 37°C; however at 10°C the Null mutant was capable of attachment but only formed dense biofilms when inoculated at high OD600. This occurred independently of division. Preliminary experiments suggest that a dual-fluorescent reporter system can be used to identify persister formation, based on visualising gene expression following sanitizer treatment.

Conclusions: We provide the first evidence for involvement of cspA paralogues in persister formation and implicate a role for translation.

We also show that frequency of persister formation is greatly enhanced at low temperature. This is partly dependent on cspA paralogues. Despite the inability to divide at 10°C, Null cells remain capable of attachment and dense biofilm formation.

S. Silva1, M. Negri1, M. Henriques1, R. Oliveira1, D. Williams2, J. Azeredo2; 1 Minho University, Braga, PORTUGAL, 2 Cardiff University, Cardiff, UNITED KINGDOM

Urinary tract infection (UTI) is the most common type of nosocomial infection and 80% are related to the use of urinary catheters. Furthermore, Candida spp are responsible for around 15% of UTIs and an increasing involvement of non-Candida albicans Candida (NCAC) species (e.g. C. glabrata, C. tropicalis and C. parapsilosis) is being recognised. Candida biofilms provide a persistent reservoir of infectious organisms and also exhibit elevated resistance to antifungal agents. Thus, the aim of this work was to compare both the adhesion and biofilm formation of different urinary isolates of NCAC species to silicone, in the presence of urine. Isolates of C. parapsilosis (n=2), C. tropicalis (n=2), and C. glabrata (n=2) from UTIs, together with reference strains of each species were assayed. Adhesion (2 h) and biofilm formation (4, 8, 12, 24, 48, 72 h) was performed by incubating silicone coupons with 1x10⁵ yeast/ml in artificial urine and the biofilm biomass then assessed by crystal violet staining. Hydrophobicity and surface charge of cells was...
determined by measuring contact angles and zeta potential, respectively. The number of viable cells in the biofilm was determined in 4 h and 72 h biofilms by CFU counting’s, after appropriate culturing. Biofilm structure was observed by confocal laser scanning microscopy (CLSM) after staining with Fun1 and concanavalin A-Alexa 488. The results showed that all isolates adhered to silicone in a species and strain dependent manner, with C. parapsilosis showing the lowest extent of adhesion. Furthermore, the extent of adhesion to silicone was not dependent on cell surface hydrophobicity or charge, since all the tested cells were hydrophilic with a negative zeta potential. Despite a higher number of viable cells being recovered after 72 h incubation, no significant biofilm formation was observed and CLSM showed an absence of extracellular polymeric material for all studied isolates. In summary, this work demonstrates that all tested NCAC species were able to adhere and survive on the silicone surface in the presence of urine. However in these conditions the clinical isolates were not able to form extensive biofilms. Adherence was species dependent and was not related to either cell surface hydrophobicity or charge.

A136
MUCOSAL SURFACE INFECTIONS WITH PSEUDOMONAS AERUGINOSA GACS- MUTANTS GIVE RISE TO STABLE SMALL COLONY VARIANTS, WHICH SHOW INCREASED MUCOSAL INFLAMMATION, ANTIMICROBIAL RESISTANCE AND BIOFILM FORMATION.
L. K. Nelson, M. M. Stanton, R. E. Elphinstone, J. Helwerda, R. J. Turner, H. Ceri; Biofilm Research Group, Department of Biological Sciences, University of Calgary, Calgary, AB, CANADA

Introduction: Formation of P. aeruginosa biofilms at mucosal surfaces results in numerous chronic diseases, ranging from cystic fibrosis to prostatitis. Therefore, we sought to understand how P. aeruginosa biofilms in mucosal surface infections are regulated by two-component signaling systems. The GacS/GasA two-component system in P. aeruginosa has previously been shown to govern biofilm formation and antimicrobial resistance in vitro. GacS- mutants are poorer biofilm formers and are more susceptible to antimicrobials than wild-type (WT). However, when exposed to stress in vitro, GacS- mutants give rise to stable small colony variants (IV-SCVs), which form denser biofilms and are more resistant to antimicrobials than either GacS- or WT. Consequently, we investigated the hypothesis that under the stress of an in vitro mucosal surface infection, GacS- would give rise to mucosal surface derived small colony variants (MS-SCVs). Furthermore, akin to IV-SCVs isolates, we believed that the MS-SCVs would possess enhanced biofilm formation and antimicrobial resistance.

Methods: The rat prostate served as an in vivo model of mucosal surface infections with P. aeruginosa. The prostates of anesthetized rats were infected with one of three P. aeruginosa PA14 strains: WT, GacS- or IV-SCVs. Infection and inflammation of the prostate was followed for all strains during both acute and chronic infections by: bacterial counts, evaluations of prostate gross morphology and histology, myeloperoxidase assays, and cytokine ELISAs. P. aeruginosa colonies isolated from these prostate infections were then subjected to in vitro testing of antimicrobial susceptibility using the Calgary Biofilm Device.

Results: Infections with both PA14 WT and IV-SCVs resulted in considerable bacterial colonization and inflammation in the prostate. Infections with PA14 GacS- produced two categories of infection which differed based on SCV formation. GacS- infections from which MS-SCVs were isolated displayed significantly greater bacterial colonization and inflammation than infections where no MS-SCVs were detected. Furthermore, in vitro testing of the MS-SCVs isolated from the GacS- infections showed that these SCVs were more resistant to antimicrobials than their GacS- and WT counterparts. Conclusions: These results show that similar to in vitro stress, in vivo stress initiated by the immune response causes GacS- to throw-off SCVs, which are better biofilm formers and more resistant to antimicrobials. Consequently, the appearance of MS-SCVs result in enhanced bacterial colonization and inflammation at mucosal surfaces.

B137
IDENTIFICATION OF KLEBSIELLA PNEUMONIAE GENES PROMOTING BIOFILM FORMATION BY USE OF A FOSMID LIBRARY
S. G. Stahlhut, C. Schroll, C. Struve, K. A. Krogfelt; Statens Serum Institut, Copenhagen, DENMARK

Indwelling urinary catheters are standard medical devices utilized in both hospital and nursing homes. The most frequent complication associated with indwelling urinary catheters is the development of nosocomial urinary tract infections (UTIs), known as catheter-associated UTIs (CAUTIs). Indwelling urinary catheters favour biofilm formation of uropathogens such as Klebsiella pneumoniae by providing a surface for the attachment of bacterial adhesins, thus enhancing microbial colonization and the development of biofilm. K. pneumoniae is a well-known opportunistic pathogen commonly associated with UTIs including CAUTIs as well as sepsis and pneumonia. To identify genes involved in the ability of K. pneumoniae to form biofilm, a novel method for screening was used. A clone library was constructed by cloning the K. pneumoniae genome of the clinical isolate C3091 into a fosmid vector and expressing this in an E. coli background. A total of 1,400 clones were screened by positive selection in a biofilm microtiter plate assay. Nine clones with significantly enhanced biofilm formation were identified, of these four were found to contain the type 3 fimbriae gene cluster, a well-known K. pneumoniae virulence factor and biofilm promotor. By random insertion of Tn5 transposon into the remaining 5 clones a negative selection screening were carried out and mutants decreased in biofilm formation, and the genes involved, identified. These included genes expressing proteins involved in cell envelope biogenesis, outer membrane proteins, secretory proteins and regulatory proteins. In conclusion, our screening successfully identified genes not previously associated with biofilm formation in K. pneumoniae.

C138
ANTIBIOFILM ACTIVITY OF NANOSIZED MAGNESIUM FLUORIDE
J. Lellouche, E. Kahana, S. Elias, A. Gedanken, E. Banin; Bar-Ilan university, Ramat-Gan, ISRAEL

The ability of bacteria to develop antibiotics resistances and