American Society for Microbiology | North Carolina Branch

# North Carolina American Society for Microbiology

# 2013 Meeting


## October 26, 2013

**NC ASM 2013**

| Schedule | | |
|---|---|---|
| **Preliminaries** | | |
| 8:00 | **Registration**<br>Poster and talk set-up<br>Coffee reception<br>Awards committee meeting/organization | |
| 9:00 | Eric Anderson | Welcome & Introductory comments |
| Session 1 : **Eric Anderson, Chair** | | |
| 9:15 | Ivan C. Ndamukong | *Bacteroides fragilis* abscess survival mode: Novel insights into the regulatory network of two ECF sigma factor families and their role in mediating oxidative stress responses. |
| 9:30 | Antonia C. Perez | Microbial interactions between upper airway opportunists affect bacterial resistance, and survival and persistence in vivo. |
| 9:45 | Rinu Kooliyottil | *Rhodospirillum rubrum*: A purple non-sulfur photosynthetic bacteria with diverse metabolic adaptations |
| 10:00 | Devang Upadhyay | Lab scale in-vitro mass production of entomopathogenic nematode *Heterorhabditis bacteriophora* using liquid culture fermentation technology |
| 10:15 | Sunita Singh | Modelling growth of a nisin producing lactic acid bacteria *Streptococcus lactis* NCIM 2114 by using different growth sigmoidal functions. |
| 10:30 | Yanlu Cao | The omp117 Polysaccharide Utilization locus is A Fitness Factor for *Bacteroides fragilis* During Extraintestinal Growth in the Host. |
| 10:45 | **Poster session 1** (Even numbered posters should be attended by presenters)<br>Coffee Break<br>Vendors | |
| Session 2 : **???, Chair** | | |
| 11:30 | Diana G. Wright | Inhibition of histone acetyltransferase (HAT) activity by HBZ extends beyond the p300/CBP HAT family |
| 11:45 | Justin B. Callaway | Dengue-viral source and purity determine antibody-enhanced secretion of IL-1beta by primary human monocytes |
| 12:00 | Maria C. White | Modulation of immune gene expression in human glioma cells after treatment with the histone deacetylase inhibitor valproic acid and infection with equine herpesvirus type 1 |
| 12:15 | Mageshwaran Vellaisamy | Unravelling the mechanism of biodegradation of gossypol by fungal cultures |
| 12:30 | Kyle A. Murrah | Adenovirus promotes *Streptococcus pneumoniae* middle ear infection in the chinchilla model of otitis media |
| 12:45 | **Lunch** | |
| Session 3 : **???, Chair** | | |
| 1:30 | David A. Martinson | Irr is the transcriptional regulator that controls gene expression in response to intracellular iron levels in *Brucella abortus* |

| | | |
|---|---|---|
| 1:45 | Walter Patterson | Observations of *Galleria mellonella* positions in response to bioluminescence produced by *Photorhabdus luminescens* |
| 2:00 | Crystal Redfern | BpsR functions as a dual activator and repressor of *Bordetella* gene expression. |
| 2:15 | Tridib Ganguly | The *Bordetella pertussis* Bps Polysaccharide Enhances Lung Colonization by Conferring Protection from Complement-mediated Killing |
| 2:30 | Marcela Kokes | Host and bacterial factors mediate cytoskeletal rearrangements at the surface of the *Chlamydia trachomatis* pathogenic vacuole |
| 2:45 | **Poster session 2** (Odd numbered posters should be attended by presenters)<br>Coffee Break<br>Vendors | |
| 3:30 | **Jennifer Miller**<br>**NC Invitational Talk** | Modulation of *Leptospira interrogans* infection by the anti-inflammatory cytokine IL-10. |
| 4:00 | **Intermission**<br>Coffee break<br>Vendors<br>Awards committee meeting | |
| **Plenary session : Eric Anderson, Chair** | | |
| 4:15 | **Joseph A. Krzycki**<br>**ASM Branch Lecture** | The 22nd amino acid: Pyrrolysine. |
| **Postscript** | | |
| 5:15 | Eric Anderson | Concluding remarks<br>Awards |
| 5:30 | Jim Brown | Business meeting<br>Officer election |
| 6:00 | **Adjournment** | |

## Sponsors & Vendors:

East Carolina University

The American Society for Microbiology

NC Academy of Science

BioExpress

1.1) *Bacteroides fragilis* abscess survival mode: Novel insights into the regulatory network of two ECF sigma factor families and their role in mediating oxidative stress responses.

Ndamukong I.C., Palethorpe S., Parker A. and Smith C.J.
East Carolina University, Brody School of Medicine, Department of Microbiology and Immmunology

The *Bacteroides* species are an important component of the gut microbiota. In the colon, they maintain an intricate symbiotic relationship with their host. When Bacteroides escape the gut into more aerated sites, like the peritoneal space, significant pathological states such as abscess formation and bacteremia develop. Aerotolerance has been linked to a robust oxidative stress response which in turn is necessary for maximal virulence in a mouse intra-abdominal abscess model. A large number of transcription regulators including a set of about 15 structurally diverse extracytoplasmic function (ECF) sigma factors are induced by oxidative stress. Two of these, EcfO and EcfOF, were used as models of ECF sigma factor activity.
EcfO belongs to the ECF21 class of ECF sigma factors and experiments showed that EcfO and its anti-sigma factor, Reo, were important for resistance to oxidative stress. EcfO controls a regulon of novel lipoproteins whose distribution in nature is restricted to members of the Bacteroidetes phylum. Three of these EcfO target genes encode members of a NigD family of proteins, first described to be associated with a locus producing the bacteriocin nigrescin, in *Prevotella nigrescens*. Analysis of mutant strains devoid of these set of proteins suggest that they work together to resist free radical stress produced by menadione. We show that these proteins are glycosylated in-vivo, and are targeted to the cell membrane. A yeast two hybrid assay with a genomic DNA fusion library was employed to identify a protein interaction network of these EcfO response proteins.
EcfOF belongs to a previously uncharacterized class of ECF sigma factors, ECF114. All three members of this class are inducible under oxidative stress in B. fragilis. One putative antisigma factor shows specific interaction with all the members of the ECF114 class. A microarray gene expression approach was used to identify the regulon of EcfOF.

---

1.2) Microbial interactions between upper airway opportunists affect bacterial resistance, and survival and persistence in vivo.

Antonia C. Perez and W. Edward Swords
Department of Microbiology and Immunology, Wake Forest University School of Medicine

Otitis media (OM), or middle ear inflammation, is the most common pediatric disease for which children visit the doctor, antibiotics are prescribed, and parents miss days at work to care for a sick child. Epidemiological data from children with chronic/recurrent OM suggest that these infections are polymicrobial in nature, which may also have an impact on bacterial persistence and resistance to antibiotic treatment. In this study, we used in vitro static biofilm assays and rodent infection models to assess the role(s) of quorum sensing and beta-lactamase production on survival and persistence in polymicrobial infections of Streptococcus pneumoniae and Moraxella catarrhalis. Consistent with prior work, beta-lactamase production played a significant role in protecting S. pneumoniae from beta-lactam killing in vitro. While M. catarrhalis was more resistant to macrolide killing in polymicrobial biofilms, we found that this effect was quorum sensing-independent. An increase in the biomass of M. catarrhalis and change in the composition of extracellular matrix material could account for this observed protection in polymicrobial biofilms. On the other hand, we found that quorum sensing enhanced early colonization and played a key role in middle ear invasion in polymicrobial infections in vivo. In conclusion, interactions within polymicrobial communities of M. catarrhalis and S. pneumoniae significantly impact resistance to antibiotic treatment and bacterial persistence in vivo.

1.3) *Rhodospirillum rubrum*: A purple non-sulfur photosynthetic bacteria with diverse metabolic adaptations

Rinu Kooliyottil, Floyd Inman III and Len Holmes
Department of Chemistry & Physics, Sartorius stedim Biotechnology Laboratory, Biotechnology Research and Training Center, UNC Pembroke

*Rhodospirillum rubrum* is a Gram-negative, mesophilic, motile bacterium belonging to the group of α-proteobacteria. *R. rubrum* is a chemophotoautotroph that can undergo alcoholic fermentation, aerobic respiration and photosynthesis. *R. rubrum* grows anaerobically in the dark by fermentation of sugars or in the presence of appropriate electron acceptors by energy-linked anaerobic respiration under anoxygenic conditions. Furthermore, fructose can be fermented without the addition of accessory oxidants. However, the bacterium requires the initial presence of bicarbonate prior to fermentative growth. *R. rubrum* is also capable of photosynthesis; however, molecular oxygen is not a final end product. The reaction center of *Rhodospirillum* cannot extract electrons from water; however, other inorganic and organic molecules within their immediate environment can be used as electron donors. These bacteria produce carotenoid and bacteriochlorophyll pigments that are found in intracellular membrane vesicles known as chromatophores. The pigments are capable of capturing light to produce chemical energy. Additionally, these photosynthetic pigments are responsible for the reddish pigmentation of *R. rubrum* during photosynthesis. The metabolic diversity of this bacterium makes it a suitable candidate for various applications. For example, *R. rubrum* can be exploited to produce H2 in presence of carbon monoxide (CO) which is a direct result from the expression of an enzymatic system responsible for the oxidation of CO. In addition, *Rhodospirillum* is capable of producing biodegradable polymers (i.e. polyhydroxybutyrate) in an economically feasible way by utilizing inexpensive substrates (i.e. vinegar). *R. rubrum* can also fix and convert atmospheric nitrogen gas to ammonia; which may ultimately play a major role in plant nutrition and possible biogeochemical cycling of nitrogen. This report emphasizes the metabolic adaptations of *R. rubrum* in various growth conditions.

1.4) Lab scale in-vitro mass production of entomopathogenic nematode *Heterorhabditis bacteriophora* using liquid culture fermentation technology

Devang Upadhyay, Floyd Inman III and Len Holmes
Department of Chemistry & Physics, Sartorius stedim Biotechnology Laboratory, Biotechnology Research and Training Center, UNC Pembroke

The present study describes mass production of the entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* and its bacterial symbiont *Photorhabdus luminescens* utilizing an in-vitro, monoxenic liquid culture. EPNs were successfully cultured in the three different bioreactor working volumes of 1.5, 4 and 7 liters with final nematode yields of ≥ 104 infective juveniles (IJ) ml-1. During the mass production process, liquid nematode media was conditioned with the bacterial symbiont 24 hours prior to nematode inoculation. Subsequently, inoculated infective juveniles developed into self-fertilizing hermaphrodites within three days and eventually produced offspring. Maximum nematode densities were obtained seven days post-nematode inoculation. All three working volumes (1.5, 4 and 7 liters) produced final nematode yields of 45,666 ± 2081 IJs/mL, 41,733 ± 2193 IJs/mL and 39,000 ± 2000 IJs/mL, respectively. In vitro scale-up technology can be further optimized for production of this attractive biocontrol agent by further improving media formulation, process parameters, bioreactor design and inoculation times that will maximize the formation of infective juveniles.

1.5) Modelling growth of a nisin producing lactic acid bacteria *Streptococcus lactis* NCIM 2114 by using different growth sigmoidal functions.

Sunita Singh, Gupta Sangeeta, Singh KN and Holmes Leonard.
Department of Chemistry and Physics, UNCP, NC USA

Nisin is produced as a primary metabolite by the lactic acid bacteria *Streptococcus lactis* NCIM 2114 in its exponential growth phase. The aim of the study was to evaluate differences (if any) in the sigmoidal functions of growth in *S. lactis*. The growth of *S. lactis* in MRS medium was recorded as absorbances at wavelength of 600 nm in 25 hrs of growth cycle. This represented the cell mass and was used to plot the modified exponential curve generally given by the basic equation Ut = a+ bct as followed for sigmoidal growth curve, fitted using three different sigmoidal functions for bacterial growth, where t was time series values at time 't'. The mathematical parameters a, b, c and d were constants and were calculated to fit the Logistic, Gompertz and Richards models. The model equations derived were based on the respective equations for the three functions to model bacterial growth. The R squared values obtained on the Logistic, Gompertz and Richards models so derived were 0.830, 0.817 and 0.836 respectively and did not differ significantly from each other. Gompertz model {y = a.exp[ - exp(b - cx)] } was used for re-parameterization of the mathematical parameters (a, b, c). The lag period '$\lambda$' of growth of S. lactis was calculated (as the t-axis intercept of the tangent through inflecton point) on the derived equation: $\lambda = [(b-1)/c]$ as 3.17 h. The maximum specific growth rate 'μm' was critical to the production of nisin by the producer bacteria in an exponential growth phase. The 'μm' of S. lactis was calculated on the derived equation: μm = ac/e ('e' exp value is 2.71828) as 0.854 h-1.

---

1.6) The omp117 Polysaccharide Utilization locus is A Fitness Factor for *Bacteroides fragilis* During Extraintestinal Growth in the Host.

Yanlu Cao, Michael A. Reott, Edson Rocha, C. Jeffrey Smith
Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University

*Bacteroides fragilis* is a Gram-negative obligate anaerobe which normally colonizes the human colon as a member of the indigenous microbiota. When limited to the intestinal lumen, *B. fragilis* serves many beneficial roles. However, *B. fragilis* is the most frequently isolated organism from anaerobic infections and when translocated to a normally sterile body site, such as the peritoneal cavity, it can cause infections such as peritonitis and intra-abdominal abscess, which can lead to significant mortality.

To search for factors that may contribute to virulence, an in vivo abscess model was developed in rats. Gene expression profiling showed that the polysaccharide utilization operon, *omp117*, is highly expressed in our abscess model when compared to expression in vitro. Since the ability to catabolize dietary or host derived glycans as is a critical factor for *Bacteroides* to grow in the colon, it was hypothesized that the polysaccharide utilization operon *omp117* is a fitness factor for B. *fragilis* that promotes survival in extraintestinal sites such as an intra-abdominal abscess. Preliminary data show that a mutant lacking the omp117 operon is significantly impaired for growth in the rat model compared to the wild type. In an in vivo competition assay, in which the growth of wild type and *omp117* mutant co-cultured in our rat model were compared, the wild type outgrew the *omp117* mutant rapidly. These results indicate that the *omp117* operon is a fitness factor for extraintestinal growth of *B. fragilis* in the animal host.

---

2.1) Inhibition of histone acetyltransferase (HAT) activity by HBZ extends beyond the p300/CBP HAT family

Diana G. Wright, Nicholas Polakowski, and Isabelle Lemasson
Department of Microbiology and Immunology East Carolina University, Brody School of Medicine, Greenville, NC 27834, USA

We previously reported that HTLV-1 basic leucine zipper factor (HBZ) interacts with the cellular coactivator p300 in cells derived from ATL patients. We further determined that HBZ directly binds to the histone acetyltransferase (HAT) domain of both p300 and its homologue CBP. HAT activity transfers an acetyl group to lysine residues on histone tails and transcription factors to generally upregulate transcription. We observed that the HBZ interaction with the HAT domain of p300/CBP inhibits acetylation of histones and of the tumor suppressor p53. In this study, we wanted to determine whether inhibition of HAT activity was limited to p300/CBP or extended to other HAT families. We focused on the GCN5/ p/CAF and MYST HAT families. We found that HBZ co-immunoprecipitates with both p/CAF and HBO1. These data support a recent finding that HBZ interacts with HBO1 in a yeast two-hybrid assay. Through in vitro HAT assays using recombinant proteins we found that HBZ inhibits acetylation of histone H3 and histone H4 by p/CAF and HBO1, respectively. Furthermore, HBZ reduces acetylation of p53 by p/CAF. Since both p300 and p/CAF acetylate p53 to increase its DNA-binding activity, we performed quantitative RT-PCR to evaluate expression of the p53 target genes, GADD45A and NOXA. We observed reduced mRNA levels of these genes when cells expressed HBZ. Overall these results suggest that HBZ inhibits the HAT activity of coactivators from different HAT families to contribute to transcriptional deregulation in HTLV-1 infected cells.

2.2) Dengue-viral source and purity determine antibody-enhanced secretion of IL-1beta by primary human monocytes

Justin B. Callaway[1], Douglas G. Widman[2], Scott A. Smith[4,5], Karen P. McKinnon1, Dirk P. Dittmer[1,3], James E. Crowe, Jr[4,6,7], Aravinda M. de Silva[1], Jenny P.-Y. Ting[1,3]
[1]Department of Microbiology and Immunology, [2]Department of Epidemiology, and [3]Lineberger Comprehensive Cancer Center, Institute of Inflammatory Diseases and Center for Translational Immunology, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [4]The Vanderbilt Vaccine Center and the [5]Departments of Medicine, [6]Pathology, Microbiology and Immunology, and 7Pediatrics, Vanderbilt Medical Center, Nashville, TN, USA.

A small portion of patients ill with dengue virus (DENV) develop severe dengue, with potential hypovolemic shock due to vascular leakage. With four DENV serotypes, severe dengue is typically associated with a secondary infection with a serotype differing from the primary infection, leading to theories that cross-reactive immunity enhances disease. Antibody-dependent enhancement (ADE) of DENV infection is one theory thought to contribute to severe dengue, in which pre-existing antibodies cause antibody-mediated viral uptake into Fc-receptor-bearing cells, potentially contributing to the creation of a “cytokine storm.” Inflammatory cytokine IL-1beta, matured by the activation of an inflammasome complex, is elevated in many severe dengue patient profiles and has known in vitro effects on vasculature. With little known about the production and contribution of IL-1beta to severe dengue, we hypothesized that ADE of DENV into primary human monocytes causes enhanced IL-1beta secretion. Consistent with the literature, anti-DENV antibodies caused greatly enhanced viral replication in primary human monocytes. Also, ADE of full DENV supernatant produced by mosquito cells greatly enhanced the secretion of IL-1beta, which was rapid and independent of viral replication. Analysis of inflammasome activation showed that ADE caused elevated IL-1beta secretion primarily via induction of pro-IL-1beta formation, which was subsequently matured by constitutive inflammasome activation. Interestingly, when testing full DENV supernatant produced by mammalian Vero cells, another common method of DENV preparation, we found ADE to be completely dispensable for IL-1beta secretion. Further analysis indicated that Vero cells concurrently produced a non-virion-associated inflammatory moiety, as clearance of virions from the supernatant only partially reduced IL-1beta. Purifying Vero-produced DENV virions made ADE necessary for elevated IL-1beta secretion. While ADE of DENV infection greatly enhances IL-1beta secretion via pro-IL-1beta formation, the source and purity of DENV preparations should be carefully considered for studies of the immune response to DENV.

2.3) Modulation of immune gene expression in human glioma cells after treatment with the histone deacetylase inhibitor valproic acid and infection with equine herpesvirus type 1

Maria C. White and Arthur R. Frampton, Jr.
Department of Biology and Marine Biology, University of North Carolina at Wilmington

Glioblastoma multiforme, the most frequently occurring primary brain tumor in humans, is highly refractory to conventional modes of treatment. As such, viral oncolytic therapy has recently become an alternative approach against this cancer type. Our group has previously shown that the cytolytic animal virus equine herpesvirus type 1 (EHV-1) can effectively infect and kill a panel of human glioma cell lines, and that the histone deacetylase inhibitor valproic acid (VPA) strongly synergizes with EHV-1 and leads to significant augmentation in viral entry, replication, cell to cell spread, and cell lysis. We are currently exploring the mechanism(s) by which VPA enhances the oncolytic potential of the virus. Using a PCR array containing primer sets for 84 genes involved in the human innate and adaptive immune responses, we show that VPA pre-treatment of glioma cells results in significant downregulation of the Toll-like receptor 4 (TLR4) pathway in these cells. Activation of the TLR4 pathway leads to the production of antiviral cytokines such as interferon and ultimately results in the establishment of an antiviral state in cells. Inhibition of this pathway may allow for a more favorable environment for viral infection and cell lysis. Currently, single gene qPCR assays are being performed to validate the PCR array results and pharmacological inhibitors of the genes of interest are being tested for synergistic capabilities with the virus.

---

2.4) Unravelling the mechanism of biodegradation of gossypol by fungal cultures

Mageshwaran Vellaisamy[1,3], A A Kathe[1], A Shaikh[1], Meetali G Chinnkar[1], A Arputharaj[1], Veda Krishnan[2], Floyd Inman III[3] and Len Holmes[3]
[1] Central Institute for Research on Cotton Technology, Matunga (E), Mumbai, India– 400019, [2] Indian Agricultural Research Institute, Pusa, New Delhi – 110012, [3] Department of Chemistry & Physics, Sartorius stedim Biotechnology Laboratory, Biotechnology Research and Training Center, UNC Pembroke

Gossypol is a toxic polyphenolic compound present in cottonseed which limits its use in non-ruminants animal feed. Even low concentration of free gossypol i.e., (0.01% to 0.1%) in feed causes toxicity to poultry. Hence the reduction of gossypol content in cottonseed cake before using as feed for non-ruminants is a need of the hour. Among the different gossypol detoxification methods, microbial method of detoxification is a promising method since microorganisms degrade gossypol unlike the other methods which do mere inactivation. The results on our previous experiments showed that the culture combinations viz., *Pleurotus sajor-caju* and *Saccharomyces cerevisiae* MTCC 6933 and *Saccharomyces cerevisiae* and *Candida tropicalis* detoxify gossypol in cottonseed cake during solid state fermentation process. In this study, the mechanism of biodegradation of gossypol in mineral medium using the above mentioned culture combinations were examined. Mineral medium containing gossypol acetic acid as a sole carbon source was inoculated with fungal cultures. Incubation of 48 hours resulted in maximum degradation of gossypol (72 %) in *P. sajor-caju* and *S. cerevisiae* 6933 combination and (53.3 %) in *S. cerevisiae* and *C. tropicalis* combination. The characterization of biodegraded gossypol samples using UV-Vis spectrum, Thin Layer Chromatography, FT-IR and HPLC showed that there is a sharp decrease in gossypol concentrations, in fungal cultures inoculated samples. To conclude, the present study revealed that these fungal cultures degrade gossypol for its growth and multiplication and also supports the results of our previous experiments. The identification of enzymes or metabolites produced during biodegradation of gossypol would be interesting area for further studies.

---

2.5) Adenovirus promotes *Streptococcus pneumoniae* middle ear infection in the chinchilla model of otitis media

Kyle A. Murrah, Roberta Lynn Turner, David Ornelles, and W. Edward Swords
Department of Microbiology & Immunology, Wake Forest School of Medicine

Viral upper respiratory tract infections are a major risk factor for development of otitis media (OM) and children with adenoviral infections have been reported to be 3-4 times more likely to experience OM. Bacterial agents of OM normally reside in the nasopharynx until they ascend through the eustachian tubes to establish infection in the middle ear. We hypothesized that adenovirus promotes bacterial ascension into the middle ear through the disruption of normal mucociliary function in the eustachian tubes due to inflammation-induced changes.
We established an intranasal infection model with type 5 adenovirus in the chinchilla. Adenovirus was detected in the eustachian tubes at seven days post-inoculation. We also established a chinchilla intranasal infection model for Streptococcus pneumoniae, the most commonly isolated bacterial species from children with OM. We found that *S. pneumoniae* strain EF3030 persisted through seven days post-inoculation in the nasopharynx; however, only about thirty percent of ears were culture-positive for *S. pneumoniae* at any given time point after inoculation.
When animals were inoculated with *S. pneumoniae* seven days after inoculation with adenovirus, there was a significant increase in the proportion of *S. pneumoniae* culture-positive ears. The adenovirus mutant deleted of the E3 region, dl327, induces more extensive inflammation in the cotton rat model. We used dl327 to test the hypothesis that adenoviral infection-induced inflammation promoted bacterial ascension. Interestingly, infection with the adenovirus mutant dl327 induced a comparable degree of bacterial ascension into the middle ear as did infection with the wild-type virus. In contrast, infection with the adenovirus mutant H5wtΔpTP, which expresses early gene products but does not direct viral genome replication, resulted in less extensive middle ear infection compared to wild-type adenovirus. Together, these results suggest that factors other than inflammation contribute to the role of adenoviral infection in promoting middle ear disease by *S. pneumoniae*.

---

3.1) Irr is the transcriptional regulator that controls gene expression in response to intracellular iron levels in *Brucella abortus*

David A. Martinson, and R. Martin Roop II
Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University, Greenville, NC 27834.

As an intracellular pathogen, *B. abortus* must overcome iron sequestration in the host cell by utilizing highly efficient iron transport systems. These systems must be tightly regulated, however, as excess intracellular iron is toxic to the bacterial cells. Most of the alpha-proteobacteria rely on a transcriptional regulator known as the iron response regulator (Irr) to control the expression of their iron metabolism genes. In these bacteria, Irr serves as an activator of genes involved in iron acquisition and a repressor of genes encoding for products that require high levels of iron for their function or serve as iron storage proteins. An isogenic *B. abortus irr* mutant produces significantly less siderophore when grown under iron limiting conditions compared to the parent strain. The *irr* mutant is also significantly less sensitive to the iron requiring antibiotic streptonigrin and exhibits a slower rate of radioactive iron uptake than the parent strain, indicating that the *irr* mutant is unable to efficiently internalize iron. Microarray analysis and real-time RT PCR have been used to show that essentially all of the known genes encoding for iron uptake systems are mis-regulated in the *irr* mutant strain, along with the genes encoding for cytochrome biosynthesis proteins and iron storage proteins. The iron responsive activity of the *B. abortus* Irr protein is unique, in that when intracellular iron levels are high, Irr is degraded and it can no longer function as a transcriptional regulator. We have experimentally determined that an internal HXH heme binding motif that is highly conserved among the alpha-proteobacteria is required for iron dependent degradation of Irr in *B. abortus*. We are presently exploring the mechanism behind the HXH dependent iron responsive degradation of Irr in *B. abortus* in an effort to better understand how Irr coordinates the expression of iron metabolism genes.

3.2) Observations of *Galleria mellonella* positions in response to bioluminescence produced by *Photorhabdus luminescens*

Walter Patterson, Floyd Inman III and Len Holmes
Department of Chemistry and Physics, Sartorius stedim Biotechnology Laboratory, Biotechnology Research and Training Center, UNC Pembroke

*Photorhabdus luminescens* is a Gram-negative, bioluminescent bacterium that is symbiotically associated with the entomoparasitic nematode *Heterorhabditis bacteriophora*. *H. bacteriophora* is able to infect a wide array of larval insect hosts insect such as *Galleria mellonella*. Once within the insect hemolymph, *P. luminescens* is expelled from the gut of *H. bacteriophora*. As *P. luminescens* proliferate within the insect, production of digestive enzymes, toxins and antimicrobial compounds that support the growth, development and reproduction of its nematode partner in vivo. The role of bioluminescence production in *P. luminescens* is yet to be known, although a possible explanation is that bioluminosity is used for the attraction of potential host insect larvae. Observations of the attracting effect of bioluminescence on larval stages of *G. mellonella* were relatively measured by the position of the larval head and direction. This study has noted a loose, negatively proportional correlation between the intensity of luminosity, reported as relative luminosity units (RLUs) of a culture of *P. luminescens*, and the final head position of *G. mellonella* larvae after specific exposure times. Results also exhibit a negatively proportional trend in the variance and standard deviation of the results as RLUs increase within bacterial cultures. The results of this study support the idea that bioluminescence of *P. luminescens* may be an insect attractant to easily allow *H. bacteriophora* to infect a new host.

---

3.3) BpsR functions as a dual activator and repressor of *Bordetella* gene expression.

Crystal Redfern, Tridib Ganguly, Matt S Conover, Tracy L Nicholson and Rajendar Deora.
Department of Microbiology, Wake Forest University

*Bordetella* are Gram-negative respiratory pathogens of animals, birds, and humans. A hallmark feature of some *Bordetella* species is their ability to efficiently survive in the respiratory tract even after vaccination. Our overall hypothesis is that establishment of colonization and persistence is enhanced by formation of biofilms in the respiratory tract. *Bordetella bronchiseptica* and *Bordetella pertussis* form biofilms on abiotic surfaces and in the mouse respiratory tract. The Bps exopolysaccharide is a critical determinant for biofilm formation and the survival of *Bordetella* in the murine respiratory tract. Regulatory pathways controlling biofilm development and polysaccharide gene expression are poorly understood in *Bordetella*. We previously showed that BpsR, a MarR like regulator repressed the expression of Bps and inhibited biofilm development. DNAse I footprinting assays have identified regions protected by BpsR on *bpsA-D*. The protected region harbors a highly unusual complex array of symmetry elements in the form of inverted, complementary inverted, mirror-like direct repeats and nine repeats of a loosely conserved hexameric sequence with a consensus G/C(A/T)4G/C. Single round in vitro transcription assays showed that BpsR is sufficient to repress transcription from the *bpsA-D* promoter. Transcriptome profiling revealed that BpsR functions as global regulator and can both activate and repress gene expression. The results of this study elucidate one mechanism of biofilm regulation and that BpsR influences the broader transcriptome of *Bordetella* beyond the bpsA-D locus. Results from this study should also provide insight into regulation of large gene networks in which transcription factors must play both positive and negative roles.

---

3.4) The *Bordetella pertussis* Bps Polysaccharide Enhances Lung Colonization by Conferring Protection from Complement-mediated Killing

Tridib Ganguly, John B. Johnson1, Griffith D. Parks and Rajendar Deora
Department of Microbiology and Immunology Wake Forest School of Medicine, Medical Center Blvd., Winston-Salem, NC, 27157.

*Bordetella pertussis* is a human-restricted Gram-negative bacterial pathogen that causes whooping cough or pertussis. Pertussis is the leading vaccine preventable disease that is resurging in the USA and other parts of the developed world. There is an incomplete understanding of the mechanisms by which *B. pertussis* evades killing and clearance by the complement system, a first line of host innate immune defense. The present study examined the role of the Bps polysaccharide to resist complement activity in vitro and in the mouse respiratory tract. The *bps* mutant was more sensitive to serum and complement mediated killing than the WT strain. This heightened sensitivity was due to enhanced deposition of complement proteins and the formation of membrane attack complex, the end product of complement activation. Bps was sufficient to confer complement resistance as evidenced by a Bps-expressing *E. coli* being protected by serum killing. The *bps* mutant strain colonized the lungs of complement-deficient mice at higher levels than that observed in C57Bl/6 mice. These results reveal a previously unknown interaction between Bps and the complement system in controlling *B. pertussis* colonization of the respiratory tract. These findings also make Bps a potential target for the prevention and therapy of whooping cough.

---

3.5) Host and bacterial factors mediate cytoskeletal rearrangements at the surface of the *Chlamydia trachomatis* pathogenic vacuole

Marcela Kokes and Raphael H. Valdivia
Department of Molecular Genetics and Microbiology, Center for Microbial Pathogenesis
The obligate intracellular bacterial pathogen *Chlamydia trachomatis* resides within a membrane-bound vacuole (“inclusion”). A cytoskeletal cage of actin and intermediate filaments surrounds the inclusion, and disruption of this cage leads to increased exposure of bacterial products to the host cell’s innate immune receptors and increased expression of pro-inflammatory cytokines.

To identify *Chlamydia* factors involved in cytoskeletal cage assembly at inclusions, we screened an arrayed library of chemically-mutagenized *C. trachomatis* strains for changes in F-actin assembly at inclusions and identified two mutants with altered F-actin cages. Using new genetic tools developed for *Chlamydia*, we determined that loss of F-actin cage assembly at the inclusion is caused by a mutation in an inclusion integral membrane protein, renamed INAC (Inclusion membrane protein for actin cages). Re-introduction via transformation of wild-type INAC under its endogenous promoter on a chlamydial plasmid rescued F-actin cage formation. Enhanced F-actin cages is caused by mutation of a unique *Chlamydia* gene with weak domain homology to a histone acetyl transferase.

In parallel, we sought to define the host factors contributing to F-actin cage formation around the inclusion. Previous studies determined that RhoA is necessary for the formation of F-actin cages at inclusions and that EGFP-RhoA localized to the inclusion periphery. We identified proteins that co-immunoprecipitated with an EGFP-tagged RhoA variant specifically during *Chlamydia* infection. Most of these cytoskeletal proteins localized around inclusions and co-localized with F-actin cages when tagged and expressed and we have confirmed the same endogenous localization of a few. Many identified proteins are structural and interact directly with actin filaments, often in the context of adhesion. Taken together, these data are consistent with a model in which INAC recruits large adhesion-like complexes to play structural or signaling roles fundamental in F-actin cage formation."

---

**NC Invited Speaker**

**Jennifer Miller**

**Associate Professor of Microbiology at**

**North Carolina State University**

**Modulation of *Leptospira interrogans* infection by the anti-inflammatory cytokine IL-10**

My laboratory is interested in the mechanisms utilized by bacterial pathogens to interact with the host's immune system to cause disease. Specifically, my research focuses on dissecting the host inflammatory pathways activated by two pathogenic spirochetes, *Borrelia burgdorferi* and *Leptospira interrogans*. *B. burgdorferi* is the causative agent of Lyme disease, a debilitating, multi-system disorder whose symptoms, including arthritis, are largely attributed to an over-exuberant host response. My laboratory utilizes tissue culture and mouse models to examine both the bacterial and host-derived mechanisms that trigger the inflammatory pathologies suffered by Lyme disease patients. The zoonotic pathogen *L. interrogans* is the causative agent of leptospirosis. Both guinea pigs and hamsters are utilized as animal models of acute, lethal leptospirosis, whereas experimental injection of mice and rats results in asymptomatic chronic carriage in the renal tubules and shedding of the bacteria into the environment within urine. Humans become infected with these bacteria via abraided skin or mucous membrane contact with contaminated rodent urine. Sepsis and multi-organ failure due to pulmonary hemorrhage, renal or liver failure are the major complications of leptospiral infection. Elucidation of the host genetic and immunological factors that control disease susceptibility versus resistance is critical for the development of a safe, effective human leptospirosis vaccine. My laboratory is currently assessing whether induction of the anti-inflammatory cytokine IL-10 within mouse organs following *L. interrogans* infection prevents the development of inflammation. I will discuss our recent data on the immunomodulatory role of IL-10 within *L. interrogans*-infected kidneys.

**Keynote Address**

**Joseph A. Krzycki**

**Professor at The Ohio State University**

***The 22nd amino acid : Pyrrolysine***

As an NSF pre-doctoral fellow, Krzycki did seminal work on the major route found in nature for methane formation by microbes called methanogens. As a postdoc, he identified a methanogen DNA binding protein that was later found to be related to histone proteins that organize human genes. As a professor, he focused on understanding routes for methane formation from abundant methylated compounds found in natural environments. His lab has identified the proteins and genes essential for methane formation from five such substrates. They found some genes were interrupted by apparent "stop" signals, an observation which led to discovery of the 22nd genetically encoded amino acid, pyrrolysine. Pyrrolysine was recognized by Discover magazine as among the top discoveries of 2002. The Krzycki lab showed that methanogenesis from nitrogenous compounds required genetic code expansion to include pyrrolysine, and that this was mediated by as few as five genes. These five genes can reprogram the genetic code of E. coli to include pyrrolysine. Recently, the Krzycki lab described the complete route of pyrrolysine biosynthesis from the simpler amino acid, lysine. Dr. Krzycki has been named a Distinguished Scholar of the Ohio State University and is a member of the American Academy of Microbiology.

| | |
|---|---|
|  | Dr. Krzycki's plenary lecture is supported by the **ASM Branch Lectureship Program**. The ASMBL program, formerly known as the Waksman Foundation for Microbiology Lectures Program, allows ASM branches to secure outstanding lecturers for their scientific meetings. The program has been operating for over 40 years, and lecturers continue to enhance scientific meetings at the local level. |

**Poster Presentations**

| | | |
|---|---|---|
| 1 | Ahmed Elhassanny | FeuPQ is a potential transcriptional regulator of the acid-responsive expression of *ftrABCD*, the ferrous iron specific transporter encoding genes in *Brucella abortus* 2308 |
| 2 | Akarsh Manne | Discovery and characterization of a *Borrelia burgdorferi* protein encoding the unique collar structure. |
| 3 | Anastasia Weeks | Investigation of the Immunosuppressive Function of the Vaccinia Virus Protein O1L |
| 4 | Ashley N. Wercholuk | Elucidating "Consumption:" Using fluorescent steroid probes to understand host cholesterol utilization by *Mycobacterium spp*. |
| 5 | Autumn Cheek | Antiviral Effects of Quercetin Metabolites |
| 6 | Becky A. Bentz | Characterization of glycoproteins B, D, H, and L from neurologic equine herpesvirus type 1 strains |
| 7 | Chirayu Patel | The Use of Vesicular Stomatitis Virus and Natural Products for the Treatment of Cervical Cancer |
| 8 | Elizabeth A. Novak | Determination of the effects of chemotaxis in the enzootic life cycle of *Borrelia burgdorferi*, the Lyme disease spirochete |
| 9 | Daniel Merrill | Characterization of Bacterial Population Density, Composition, and Antibiotic Resistance in College Gyms |
| 10 | Denise Aslett | Increasing Photosynthetic $CO_2$ capture in Camelina with a Synthetic Carbon Fixation Cycle Composed of Select Microbial Enzymes |
| 11 | James W. Brown | MB 360 : Scientific Inquiry in Microbiology : A course to prepare students for undergraduate research |
| 12 | Ki Hwan Moon | Role of bacterial chemotaxis and pathogenesis in *Borrelia burgdorferi* |
| 13 | M. Brandon Ludlum | Pilot Study for Carriage of MRSA in Noses and Throats of UNCW Nursing Students |
| 14 | Melanie J. Lee-Brown | Can you feel the pulse (Partnership for Undergraduate Life Science Education)? Transforming Life Science Departments |

| | | |
|---|---|---|
| 15 | Michael I. Betteken | Differential roles for *Bacteroides fragilis* iron storage proteins in vitro and in vivo |
| 16 | Saeed A. Hayek | Impact of sweet potato on the growth and enzymatic activities of lactobacilli |
| 17 | Saeed A. Hayek | Impact of metal ions on the enzymatic activity of *Lactobacillus reuteri* growing in a sweet potato based medium |
| 18 | Cullen P. O'Brien | Pyochelin Uptake as an antimicrobial target in *Pseudomonas aeruginosa* |
| 19 | Sergey Vinogradov | An Electrochemical Assay for the Rapid Determination of Antibiotic Antibiofilm Activity |
| 20 | Syed Sultan | Motility is crucial for the survival of *Borrelia burgdorferi* in the *Ixodes scapularis* midgut |
| 21 | Shayna N. Mooney | Vaccinia virus lung infection and the A35R virulence gene |
| 22 | Syed Sultan | Exploration of the mechanism of regulation of motility by Cyclic-di-GMP in *Borrelia burgdorferi* |
| 23 | Shelby Roland | Isolation and Characterization of Two Genetically Distinct *Paenibacillus larvae* Bacteriophages |

**1.** FeuPQ is a potential transcriptional regulator of the acid-responsive expression of *ftrABCD*, the ferrous iron specific transporter encoding genes in *Brucella abortus* 2308

Ahmed Elhassanny and R. Martin Roop II
Department of Microbiology and Immunology, Brody school of medicine, East Carolina University

FtrABCD is a ferrous iron specific transporter that is essential for the wild-type virulence of *B. abortus* 2308 in experimentally infected mice. Our studies have found that the expression of *ftrABCD* is responsive to low-iron conditions. This response is mediated by Irr, the predominant iron-responsive regulator in *Brucella* and the other $\alpha$-proteobacteria. These genes are also induced by exposure to acidic pH in both *B. abortus* 2308 and an isogenic *irr* mutant, indicating that the iron- and pH-responsive regulation of these genes are independent processes. This acid-responsive expression of the *ftr* locus is important because it potentially allows the brucellae to fine-tune the expression of their iron acquisition genes to adapt to the acidic environment they encounter in the endolysosomal *Brucella*-containing vesicles (eBCVs), where Fe2+ is thought to be a biological relevant iron source. However, the transcriptional regulator responsible for the pH-responsive regulation of *ftrABCD* expression in *Brucella* is currently unknown.

One strong candidate for being the acid-responsive regulator of the *Brucella ftrABCD* operon is the two-component regulator FeuPQ. FeuP and FeuQ share significant levels of amino acid homology with BqsR and BqsS, respectively, which make up the two component regulator BqsRS in Pseudomonas. BqsRS senses extracellular ferrous iron. Since acidic pH favors the stability and solubility of ferrous iron, it is possible that the expression of the *Brucella ftrABCD* is induced in response to extracellular ferrous iron instead of acidic pH per se. Interestingly, the *feuPQ* locus has been shown to be required for high affinity iron acquisition by *Rhizobium leguminosarum*, a close-phylogenetic relative of *Brucella* strains. The specific contribution of FeuPQ to iron acquisition in R. leguminosarum, however, is currently unknown. Our preliminary results show that a *B. abortus feuPQ* mutant has a defect in its capacity to use ferrous iron at acidic pH, and studies are underway to determine if FeuPQ is responsible for the low pH-responsive induction of the *ftr* locus in the parental 2308 strain.

---

**2.** Discovery and characterization of a *Borrelia burgdorferi* protein encoding the unique collar structure

Akarsh Manne, Xiaowei Zhao, Jun Liu, and MD A. Motaleb
Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville, North Carolina; Department of Pathology and Laboratory Medicine, University of Texas Health Science Center, Houston, Texas

Although motility is crucial for the infectious life cycle of *Borrelia burgdorferi*, our understanding of the spirochete's asynchronous motility is limited. Moreover, the proteins encoding the unique flagellar structures that exist only in the spirochetes are unknown. One example is the collar structure that is located in the flagellar motor revealed by cryo-electron tomography (cryo-ET). Because of its prime location in the motor, we hypothesize that the collar proteins plays a critical role in the flagellar motor assembly or rotation of periplasmic flagella. Based on bioinformatics and its location in the genome, we predicted that BB0286 is a flagellar protein. To determine its role in flagellar assembly or motility, we constructed a non-polar Δbb0286 (*flbB*) mutant that is rod-shaped and non-motile, despite the synthesis of periplasmic flagella. Additionally, cryo-ET data indicates that FlbB encodes for the collar or the base of the collar. Another remarkable phenotype observed in the Δ*flbB* tomograms is that the flagella are not oriented towards the cell body, as in the wild-type cell; instead, most flagella are coiled towards the cell pole. Because of the altered flagellar orientation, these mutants were unable to move or produce the normal flat-wave morphology. Recently, we have reported that FliL is partly responsible for normal flagellar orientation. Here we report that, by interacting with FliL, which we confirmed by immunoprecipitation, FlbB likely enforces the periplasmic flagella to orient toward the cell body, producing efficient motility and wave-like morphology. Together, our data indicate that FlbB is crucial for the spirochete's mobility and morphology. Because this structural protein is required for motility and, motility for transmission and infection, it could lead to applications in structure-based drug design to disrupt assembly, therefore blocking bacterial disseminations and preventing Lyme disease.

**3.** Investigation of the Immunosuppressive Function of the Vaccinia Virus Protein O1L

Anastasia Weeks, Gwendolyn Jones, and Rachel Roper
Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University

Poxviruses have plagued humans for millennia, most notably Variola virus, the causative agent of smallpox. More recently, Monkeypox virus (endemic in Africa) caused an outbreak in humans in the US in 2003. Due to the success of the Vaccinia virus vaccine, smallpox has been eliminated as an ongoing threat from nature, but poxviruses still pose a bioterrorism threat. Vaccinia is also currently used successfully as a vector in recombinant vaccines that target diseases such as HIV, malaria and cancer. However, Vaccinia virus retains significant virulence in mammals and is unsafe in approximately 25% of the US population. In order to understand poxvirus pathogenesis, our lab is investigating poxvirus virulence genes.

In this study, we show that the previously uncharacterized, highly conserved Vaccinia virus gene O1L is a virulence factor in mammals. Prior data from this lab indicated that the O1L protein was not required for replication, therefore we hypothesized that O1L functions in some immunomodulatory capacity. To ascertain the mechanism of action of the O1L protein, we investigated the immune response in mice infected with Vaccinia virus or O1L deletion mutant viruses. Data from an ELISA demonstrated that O1L suppresses antiviral antibody production, supporting our hypothesis that O1L is immunoregulatory. Currently we are investigating the effects of O1L on T cell activation and differentiation by intracellular cytokine staining and analysis of secreted cytokines.

Removal of immunosuppressive virulence genes may allow the creation of more safe and immunogenic vaccines. In addition, immunosuppressive viral genes have the potential to be used clinically to reduce undesirable immune responses.

---

**4.** Elucidating "Consumption:" Using fluorescent steroid probes to understand host cholesterol utilization by *Mycobacterium spp*.

Ashley N. Wercholuk, Jenna M. Thuman, Jordan Stanley, Jason Gee, Eric Anderson, William E. Allen
Department of Chemistry, East Carolina University

Current research is beginning to shed light on the molecular mechanisms that allow *Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis, to be such a successful intracellular pathogen. One area of great interest is the ability of the bacteria to modify its own metabolism during the latent stage of infection. Due to this metabolic shift, Mtb is able to utilize host fatty acids, including cholesterol, for energy and as building blocks for specific virulence factors.

Our lab has synthesized fluorescently labeled cholesterol compounds that could potentially aid in elucidating the mechanism of cholesterol intake and the initial steps in fatty acid catabolism performed by the bacteria. All of the cholesterol fluorophores were modified at the 3β-hydroxyl group of steroid ring A, preserving the original steroid scaffold and lipophilic side chain. The fluorophores are sensitive to the polarity of their surrounding environment, emitting strong green light ($\lambda_{ex}$= 401 $\lambda_{em}$= 480 nm) when encompassed in nonpolar conditions, but experience significant quenching in polar milieu. However once attached to cholesterol, the fluorophores emit blue light ($\lambda_{ex}$= 372 $\lambda_{em}$= 440 nm) while maintaining their characteristic responsiveness to environmental conditions.

Our compounds should provide answers related to steroid transporter specificity, location of the initial catabolic steps, and cholesterol metabolite toxicity in the model organism M. smegmatis. The modifications made to cholesterol may help in determining the substrate specificity of the transporter. Observed changes in the emission wavelength report alterations to fluorophore structure, whether it remains attached to cholesterol (blue light) or is cleaved (green light), while the intensity of emitted light can be used to determine if the fluorophore is in a polar or nonpolar environment. With a greater understanding of these mechanisms, it may be possible to discover an "Achilles' heel" in the Mtb metabolic network, thus providing new targets for novel anti-tuberculosis drugs.

---

**5.** Antiviral Effects of Quercetin Metabolites

Autumn Cheek, D. Henson, D. Nieman and M. Ahmed
Departments of Biology and Health, Leisure and Exercise Science, Appalachian State University

Quercetin, one of the largest classes of flavonoids found in fruits and vegetables, is attributed with the ability to inhibit the infectivity and replication of a variety of different viruses. The goal of this study was to characterize the antiviral properties of the bioactive metabolites of quercetin present in the plasma of subjects following quercetin supplementation. Healthy volunteers, randomly divided into three groups, ingested (daily) soft chews containing 1000mg quercetin (Q-ForceTM), 1000mg quercetin plus 100mg isoquercetin, 30mg eqigallocatechin 3-gallate (EGCG) from green tea extract and 100mg n3-PUFAs from fish oil (Q-Force-ImmuneTM), or placebo chews. Blood plasma samples were collected prior to and after a three week duration of supplementation. HPLC analysis results indicated that the highest concentrations of quercetin were found in the plasma of subjects who ingested Q-Force-ImmuneTM (average levels of 2362microgram/L). The antiviral properties of quercetin metabolites in plasma samples were measured by determining the ability of plasma pre- and post-supplementation to prevent killing and infection of HeLa cells by vesicular stomatitis virus (VSV). There was no difference in the ability of plasma from placebo versus quercetin supplemented individuals to prevent killing of cells by VSV. However, the plasma of subjects who ingested Q-ForceTM and Q-Force-ImmuneTM was able to delay virus infectivity at early times post-infection. These results provide evidence that quercetin metabolites in plasma may provide antiviral activity.

**6.** Characterization of glycoproteins B, D, H, and L from neurologic equine herpesvirus type 1 strains

Becky A. Bentz, Danielle R. Johnson, Angela Laube, My P. Le, Emily C. Marx, Daniel K. Olson, Alma D. Sanchez, Hayden M. Greenawalt, Jekaterina Arnette, Arthur R. Frampton
Department of Biology and Marine Biology, University of North Carolina Wilmington

Equine herpesvirus type 1 (EHV-1) causes upper respiratory disease in horses which may spread to the central nervous system, causing neurologic disease, or a pregnant uterus causing spontaneous abortion. While most horses recover from the respiratory tract infection with no long-lasting negative effects, some horses will experience a more severe disease outcome following infection. One major sequelae that may arise after infection is a neurologic disease termed equine herpesvirus myeloencephalopathy (EHM), which may result in paralysis and death. Due to the recent increase in EHM cases it is critical that the underlying molecular mechanisms by which EHV-1 causes this neurologic disease be investigated so that appropriate therapeutic interventions can be developed and delivered. Previous research has shown that glycoproteins gB, gD, gH, and gL play a role in viral entry and cell-to-cell fusion. Since not all EHV-1 strains have been shown to cause EHM, this project focuses on these four glycoproteins from the neurologically damaging strains OHIO 2003 (OH03) and Ab4 as well as the non-neurologic strains RacL11 and KyA. The four glycoproteins will be studied to examine the interaction of neurologic versus non-neurologic strains of EHV-1 with cells. This study of interaction will help identify specific viral and cellular factors that contribute to efficiency of viral entry as well as cell-to-cell spread. To evaluate the glycoproteins separately, as well as different set combinations, the individual EHV-1 glycoproteins were cloned into the mammalian expression vector pcDNA3.3-TOPO and expression was verified in mammalian cells. After each glycoprotein is successfully cloned, a series of virus entry and cell-to-cell spread assays will be performed to determine which glycoproteins contribute to these processes.

**7.** The Use of Vesicular Stomatitis Virus and Natural Products for the Treatment of Cervical Cancer

Chirayu Patel and M. Ahmed
Department of Biology, Appalachian State University

Vesicular stomatitis virus (VSV) is currently being studied as a candidate oncolytic agent due to its ability to induce apoptosis in a variety of cancer cells. Previous studies have shown that matrix (M) protein mutants of VSV, such as rM51R-M virus, act as selective anti-cancer agents by targeting cancer cells while sparing normal cells. Our goal is to promote the use of VSV for the treatment of cervical cancers. The cervical cancer cell line Siha has been shown in previous studies to be permissive to infection and killing by VSV. We hypothesized that cervical cancer lines are sensitized to VSV due to blockage of the type-1 interferon (IFN) response by human papillomavirus (HPV) oncoproteins. However, our results indicated that Siha cells retained their ability to respond to type I IFN. Furthermore, in contrast to previous studies, we observed that Siha cells were sensitive to killing by both wild-type (wt) and M protein mutant VSV (rM51R-M virus) only when infected at high multiplicities of infection. In addition, cells were more sensitive to killing by rM51R-M virus than wt VSV. We tested another cervical cancer cell line, C4-II, for its sensitivity to VSV infection and results indicated that C4-II cells were more resistant to VSV infection in comparison to Siha cells. In conclusion, our results show that cervical cancer cells exhibit resistance to VSV infection, perhaps due to the maintenance of intact antiviral responses. We are currently investigating the mechanisms of resistance in different cervical cancer cell lines and developing combination therapeutic strategies with natural compounds such as resveratrol, flavocavain B, echinacea, and quercetin, to augment VSV-induced oncolysis of cervical cancers.

---

**8.** Determination of the effects of chemotaxis in the enzootic life cycle of *Borrelia burgdorferi*, the Lyme disease spirochete

Elizabeth A. Novak, Akarsh Manne, MD A. Motaleb
Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville.

Bacterial chemotaxis systems govern the swimming behavior of cells in response to environmental chemical signals, resulting in colonization and infection. The chemotaxis system of *B. burgdorferi* is well-conserved to that of other bacteria, including *Escherichia coli*. However, the *B. burgdorferi* signaling system is more complex because its genome encodes multiple homologs of chemotaxis genes (e.g. two *cheA*, three *cheW*, three *cheY*, and two *cheB* genes). To date, only one study has shown that chemotaxis is critical for both the survival of *B. burgdorferi* in mice and for migration from infected ticks to naïve mice. The CheY protein is the response regulator in the chemotaxis two-component system, which interacts with the flagellar motor switch protein to reorient the bacteria. Previous studies on the *cheY* genes (*cheY1, cheY2*, and *cheY3*) in *B. burgdorferi* indicated that only *cheY3* is essential for chemotaxis. However, those studies were done in vitro using an avirulent clone, and B. *burgdorferi* is a parasite, circulating in two disparate hosts (*Ixodes spp*. tick and *Peromyscus leucopus* mouse). Since all three *cheY* genes were expressed in growing *B. burgdorferi* cells as well as in its natural hosts, we hypothesize that these genes are essential for detecting chemical stimuli, and, thus, are crucial for colonizing the mouse and/or the tick in addition to tick-to-mouse transmission. Preliminary data using a *cheY3* mutant constructed in the virulent background suggests that the mutant cells are non-infectious in C3H/HeN mice via needle inoculation. Currently, we are investigating the importance of the *cheY3* gene in vivo using a tick-mouse infection model. These studies are focused on elucidating the role chemotaxis plays in the enzootic life cycle of *B. burgdorferi* and may lead to the development of potential therapies aimed at blocking the transmission of the spirochetes, thereby preventing Lyme disease.

---

**9.** Characterization of Bacterial Population Density, Composition, and Antibiotic Resistance in College Gyms

Daniel Merrill, Dr. Karen Bernd
Department of Biology, Davidson College

Overuse of antibiotics is known to increase the incidence of antibiotic resistant bacteria in both laboratory and health-care settings. However, as the number of cases of community-acquired antibiotic resistant bacterial infections grows, concern surrounding the use of antimicrobials outside of those controlled settings has increased. In this study we investigate the effect of antimicrobial cleaner use on the density, composition, and antibiotic resistance of bacterial populations found in a college fitness center. Weight benches in a fitness center serving a student/faculty and staff population of men and women were swabbed and used to inoculate Tryptic Soy media. Colonies grown on Trypic Soy Agar were counted to determine population density and replicate plates on selective media to test for the presence of *S. aureus*, *P. aeruginosa*, *E. coli*, and *H. influenza*. Cultured strains were then tested for resistance to G-418 sulfate, streptomycin, ampicillin, ciproflaxin, vancomycin, methicillin, and neomycin. This work is a pilot investigation that will inform the research design of a larger study using optimized metrics to characterize microbial populations in campus fitness centers and classrooms that utilize different cleaning products and cleaning regimens.

---

**10.** Increasing Photosynthetic $CO_2$ capture in *Camelina* with a Synthetic Carbon Fixation Cycle Composed of Select Microbial Enzymes

Denise Aslett, Benjamin Bobay, Mikyoung Ji, Kai Li, Xuili Lina Caroline Smith Hannah Wapshott, Deyu Xie, P.I., Amy M. Grundena, P.I
NCSU Dept. of Plant and Microbial Biology
NCSU Dept. of Biochemistry
USF Dept. of Cell Biology, Microbiology, and Molecular Biology"

*Camelina sativa* is an excellent oil crop for biofuel production because it grows with little water and fertilizer on marginal land. To improve camelina as a dedicated biofuel plant, we are increasing its photosynthetic $CO_2$-fixation rate by introducing a novel synthetic $CO_2$-fixation cycle that will result in30% lower energetic cost than the traditional Calvin-Benson Cycle. The “SynCycle” is comprised of five microbially derived enzymes and is designed to deliver glyoxylate to an engineered photorespiratory bypass. Initial experiments were designed to demonstrate SynCycle function *in vitro*.

---

**11.** MB 360 : Scientific Inquiry in Microbiology : A course to prepare students for undergraduate research

James W. Brown Department of Biological Sciences, NC State University

We have developed a novel course, MB 360 ""Scientific Inquiry in Microbiology : At the bench"". This course is designed to solve the familiar catch-22: students find it difficult to get their initial research experience because faculty or other potential mentors are reluctant to bring completely inexperienced students into the lab. The goal of this class, then, is to prepare undergraduate students so that they are attractive candidates for internships, co-ops, REUs, and undergraduate research on campus, and so initiate the process of their becoming productive scientists in industrial, academic, or government labs.

The purpose of the course isn't to teach any particular methods or system, or familiarize students with any particular sub-discipline of microbiology, but rather to teach students about scientific questions, controls and variables, designing, preparing for and carrying out experiments, keeping a notebook, interpreting results, and presenting their findings. In other words, the pragmatic things a student has to know in order to work efficiently in a research lab regardless of discipline. The experimental system of choice for this class is the bacterial growth curve. The growth curve is a fundamental aspect of microbiology with limitless facets for exploration.

---

**12.** Role of bacterial chemotaxis and pathogenesis in *Borrelia burgdorferi*

Ki Hwan Moon and MD A. Motaleb
Department of Microbiology & Immunology, Brody School of Medicine, East Carolina University, Greenville, NC

Motility and chemotaxis were reported to be crucial for the infectious life cycle of *Borrelia burgdorferi*. However, our knowledge on how this Lyme disease spirochete achieves asynchronous motility when the bacteria undergo chemotaxis during infection is limited. The components of the chemotaxis system are highly conserved among prokaryotes, but chemotaxis in *B. burgdorferi* differs from other well-studied bacterial chemotaxis models and is much more complicated due to the presence of multiple copies of chemotaxis genes. CheD is relatively well-characterized in Bacillus subtilis and Thermotoga maritima, which plays an important role in chemotaxis by modification of methyl-accepting chemotaxis protein receptors (MCPs) by deamidation or by enhancing CheC/CheX phosphatase activity, thereby regulating the levels of the CheY response regulator. We expect that CheD in *B. burgdorferi* play crucial roles in chemotaxis. To verify the roles of CheD in *B. burgdorferi*, we constructed a *cheD* mutant and complemented strain. The mutant cells exhibit defects in chemotaxis as well as in motility. Whereas the wild-type cells run, pause/flex, and then reverse, the *cheD* mutant cells failed to reverse or flex. Based on these phenotypes, we predict that CheD likely modulate a MCP or enhance the phosphatase activity. Accordingly, we are in the process of verifying its receptor deamidase activity or the phosphatase stimulating activity. A role of CheD in host infection has not been reported in any pathogens. Our preliminary studies indicate that the *cheD* mutant has reduced infectivity compared to wild-type via needle inoculation in C3H/HeN mice. We plan to determine if the *cheD* mutant will be able to complete the infection cycle comprised of both the *Ixodes* tick and mouse. Delineating the role of CheD in *B. burgdorferi* will provide insights into not only the chemotaxis pathway of this spirochete but also its asymmetric swimming and infectious life cycle.

---

**13.** Pilot Study for Carriage of MRSA in Noses and Throats of UNCW Nursing Students

M. Brandon Ludlum, Rheanna M. McKnight, Meghan Dalziel, Cortney L. Castine, Taylor S. Morrisette, Camry L. Wagner, William M. Brock, and Kevin B. Kiser
Department of Biology and Marine Biology, University of North Carolina Wilmington

Methicillin-Resistant *Staphylococcus aureus* (MRSA) accounts for high numbers of opportunistic infections in hospitals. To begin understanding the relationship between clinical exposure and increased risk of MRSA carriage, both noses and throats of UNCW nursing students were tested for colonization of *S. aureus*. All samples were transferred to ChromAgar® plates to identify *S. aureus*. All positive outcomes were tested for antibiotic resistance. Of 65 samples collected, nearly half tested positive for *S. aureus*. Ten *S. aureus* isolates were D-Test positive, demonstrating inducible clindamycin resistance. Only one sample was positive for MRSA. This study showed with 15% of the subjects being exclusive throat carriers, the pharynx is an important testing site. With results from this pilot study, the plan is to move forward with a cohort study of incoming nursing students, in order to follow them throughout their education at UNCW.

---

---

**14.** Can you feel the pulse (Partnership for Undergraduate Life Science Education)? Transforming Life Science Departments

Melanie J. Lee-Brown  Department of Biology, Guilford College

We lose bright, qualified, visionary students every year from STEM disciplines. What will it take to bring about the necessary transformation of STEM higher education described in Vision and Change: A Call to Action? PULSE (Partnership for Undergraduate Life Science Education) was a joint effort by the National Science Foundation (NSF), National Institutes of General Medical Sciences (NIGMS) and Howard Hughes Medical Institute (HHMI) to stimulate systemic change in post-secondary, life science departments. The recommendations for transformation are based on the 2011 report Vision and Change: in Undergraduate Biology Education: A Call to Action. The PULSE Leadership Fellows are tasked with facilitating pathways to foster change in undergraduate life science education at the department level. This poster will share the PULSE work to date and will invite you to get involved toward implementing change in life science education nation-wide. How can PULSE help you and your department toward transformation?

---

**15.** Differential roles for *Bacteroides fragilis* iron storage proteins in vitro and in vivo

Michael I. Betteken, Edson R. Rocha, and C. Jeffrey Smith
Department of Microbiology and Immunology, East Carolina University

*Bacteroides fragilis* is a Gram negative obligate anaerobe and member of the normal intestinal flora of humans. When limited to the intestinal tract, *B. fragilis* performs many beneficial functions; however, in the event of damage to the intestinal lining *B. fragilis* can translocate into the oxygenated peritoneal cavity. The resulting immune response to this translocation causes peritonitis and formation of intra-abdominal abscesses. It has been demonstrated that the *B. fragilis* oxidative stress response (OSR) is required for survival in the oxygenated tissue of the peritoneal cavity during abscess formation. One aspect of the *B. fragilis* OSR is the management of intracellular concentrations of ferrous iron ($Fe^{2+}$) to prevent hydroxyl radical production. To manage this *B. fragilis* utilizes three ferritin family proteins FtnA, BfDPSL, and Dps which belong to the ferritin, bactoerioferritin/DPSL, and Dps ferritin subfamilies respectively. Previous studies have demonstrated a high level of similarity between the Dps and DpsL proteins. Therefore we rationalized that DpsL and Dps may be serving similar if not compensatory roles within the cell. To evaluate this we constructed a double Δd*ps* Δ*bfr* (BfDpsL) mutant. We found that this double mutant was considerably more sensitive to oxygen exposure and tert-butyl hydroperoxide exposure during aeration. To further evaluate the double mutant phenotype we analyzed growth in vivo using the Rat Ping-Pong ball abscess model. The results demonstrated a decreased growth rate for the double mutant when compared to WT. Competition assays were also performed using the in vivo model and it was found that the double mutant was attenuated in these experiments. Further work is needed to elucidate the roles of DpsL in vivo and to define the specific roles of Dps and DpsL within the abscess.

---

**16.** Impact of sweet potato on the growth and enzymatic activities of lactobacilli

Saeed A. Hayek, Rabin Gyawali, Aboghasem Shahbazi, Reza Tahergorabi, and Salam A. Ibrahim
Food Microbiology and Biotechnology Laboratory, 163 Carver Hall, Greensboro, North Carolina Agricultural and Technical State University, Greensboro, NC 27411, USA

The objective of this study was to investigate the impact of sweet potatoes on the growth and enzymatic activity of *Lactobacillus*. Sweet potato medium (SPM) was formed using baked sweet potato as basic component. The growth and enzymatic activities of *Lactobacillus* in SPM was compared to that in MRS. Seven strains of *Lactobacillus* were screened in SPM and MRS for α-glucosidase (EC 3.2.1.20), ß-glucosidases (EC 3.2.1.21), acid phosphatase (EC. 3.1.3.2), and phytase (EC. 3.1.3.26) activities. These activities were determined spectrophotometrically using the corresponding substrate. The growth of Lactobacillus strains was monitored using turbidity (OD 610 nm) and bacterial population (log CFU/mL). Lactobacillus strains conteue to grow in SPM and MRS at similar growth rates and reached averages of 10.98±0.49 and 10.92±0.55 log CFU/mL respectively. No significant ($p>0.05$) differences in the growth rates and bacterial populations were observed. *Lactobacillus* strains growing in SPM showed relatively higher ß-glucosidases, acid phosphatase, and phytase activities and lower α-glucosidase compared to that in MRS. Strains of *L. reuteri* showed the highest enzymatic activities of α-glucosidase, acid phosphatase, and phytase whereas *L. delbrueckii subsp. bulgaricus* showed the highest ß-glucosidases activity. Enzymatic activities showed a wide range of difference among *Lactobacillus* strains growing in the two media. Thus, sweet potatoes could serve the growth of *Lactobacillus* strains and enhance their enzymatic activity.

**17.** Impact of metal ions on the enzymatic activity of *Lactobacillus reuteri* growing in a sweet potato based medium

Saeed A. Hayek, Rabin Gyawali, Aboghasem Shahbazi, Reza Tahergorabi, and Salam A. Ibrahim
Food Microbiology and Biotechnology Laboratory, 163 Carver Hall, Greensboro, North Carolina Agricultural and Technical State University, Greensboro, NC 27411, USA

*Lactobacillus reuteri* is among the most important probiotic species with well documented characteristics including enzymatic activity. The objective of this study was to investigate the effect of metal ions on α-glucosidase (EC 3.2.1.20), ß-glucosidases (EC 3.2.1.21), acid phosphatase (EC. 3.1.3.2), and phytase (EC. 3.1.3.26) activity of *Lactobacillus reuteri* growing in a sweet potato medium (SPM). Ten mM of $Na^+$, $K^+$, $Fe^{2+}$, and $Mg^{2+}$ and 5 mM of $Mn^{2+}$, $Ca^{2+}$ were added separately to batches of 60 mL of SPM. Six strains of *L. reuteri* (CF2-7F, DSM20016, MF14-C, MM2-3, MM7, and SD2112) where then inoculated individually into SPM with metal ions. Our results showed that the presence of $Mg^{2+}$ and $Mn^{2+}$ enhanced the growth of *L. reuteri* whereas present of $Ca^{2+}$ slow down the growth. In control, the highest activity of α-glucosidase, ß-glucosidase, acid phosphatase, and phytase was obtain from *L. reuteri* DSM20016, *L. reuteri* MM2-3, *L. reuteri* CF2-7F, and *L. reuteri* DSM20016 respectively. *L. reuteri* DSM20016 showed high enzymatic activity for all tested enzymes and showed better response to metal ions compared to other strains. The addition of $Mg^{2+}$ or $Mn^{2+}$ showed the highest enhancement effect on all tested enzymes. The addition of $Mg^{2+}$ or $Mn^{2+}$ enhanced the four enzymes by averages of 43.7 and 38.8% respectively. $Na^+$, $K^+$ and $Fe^{2+}$ fallen between enhancement and slight reduction effect on the enzymatic activity of *L. reuteri*. The response of *L. reuteri* strains to metal ions was found to be a strain dependent. Thus, *L. reuteri* DSM20016 should be given more attention and $Mg^{2+}$ and $Mn^{2+}$ could be used to produce enhanced level of lactobacilli enzymes.

**18.** Pyochelin Uptake as an antimicrobial target in *Pseudomonas aeruginosa*
Cullen P. O'Brien, Caitlyn S. Daron, Kaylynn Font, Brittany A White, Robert M. Dodder, Ryshonda P. Lewis and Eric S. Anderson
Department of Biology, East Carolina University, Greenville, North Carolina

Approximately 30,000 children and adults are affected by cystic fibrosis in the United States. *Pseudomonas aeruginosa* is an opportunistic pathogen that establishes persistent biofilms in the lungs of these patients resulting in severe and often fatal respiratory infections. Like most bacteria, *Pseudomonas* requires iron and captures it from the environment using small secreted compounds called siderophores. Biosynthesis and uptake of one of these siderophores, pyochelin, is controlled using the AraC-like regulator, PchR. In this pathway, the siderophore serves as a co-activator for its own biosynthesis when bound to iron. Metabolically, this represents a very efficient system. When iron levels are low, pyochelin is produced at a basal level. If ferric iron is present, pyochelin captures the metal and returns it, signaling the presence of a viable iron source. This increases production of that specific uptake pathway. If pyochelin returns without iron, basal production continues and other acquisition systems are utilized to acquire sufficient levels of this essential metal. This pathway presents an attractive target for antimicrobial development. These antimicrobials would not only be pathogen specific, but would also stimulate their own uptake, thus increasing their efficacy even further. The net result would be an antimicrobial that is highly effective at very low concentrations.
Our work focuses on the ability of gallium-complexed pyochelin (G-Pc) to target this pathway. Gallium ($Ga^{3+}$) is a metal with an ionic radius similar to ferric iron ($Fe^{3+}$) and can be incorporated into iron binding sites in the bacterium. However, gallium is toxic, as it is not able to be reduced, a critical property for iron to act as a biological cofactor. Preliminary experiments indicate that G-Pc far more effective than gallium alone at killing liquid cultures of *P. aeruginosa* and is significantly better at disrupting established biofilms. We are currently further evaluating the effect of G-Pc on initial and established biofilm and examining the effect of G-PC on pyochelin biosynthesis and uptake.

---

**19.** An Electrochemical Assay for the Rapid Determination of Antibiotic Antibiofilm Activity
Sergey Vinogradov*, Erik Anderson#, Rickey P. Hicks*, Eli G. Hvastkovs*
# - Department of Biology, ECU, * - Department of Chemistry, ECU

Biofilms are a significant global financial and health concern[1,2]. A major contributing factor leading to antibiotic resistance is the ability of bacteria to form protective biofilms around their colonies[1,2]. In order to combat this process, safe antibiofilm antibiotics need to be manufactured and their efficacy tested[1,3,4]. A series of peptides containing unnatural amino acids has been synthesized for this purpose. These peptides feature Tic-Oic dipeptide units and multiple cationic charges that promote α-helix morphology. The peptides have previously been shown to have antimicrobial behavior[4].

To test the antibiofilm properties of the peptides, a novel electrocanalytical sensor approach as well as standard biological methods have been employed. Electrodes coated with polymers of alternating charge and capped with an anionic alginate layer were constructed following layer-by-layer methodology. The alginate layer mimics the biofilm produced by *Pseudomonas aeruginosa*. Antibiofilm properties were assayed by monitoring the electrochemical signal increase as a function of peptide exposure time as the peptides promote electrode access of an external solution-phase redox probe molecule, Fe(CN)63-. The electrochemical responses were validated by performing both bacterial inhibition and biofilm dispersion assays in 96-well plates using *Pseudomonas aeruginosa* PAO1 as the biofilm model. Overall, the electrochemical sensor results mirror those of the biological assays, which demonstrates the utility of the analytical approach to assay antibiofilm behavior rapidly while also saving valuable material in a cost-effective fashion[5].

References

1. Worthington, R J.; Richards, JJ.; Melander, C. Small molecule control of bacterial biofilms. Org. Biomol. Chem. 2012, 10, 7457-7474.
2. Costerton, JW.; Stewart, PS.; Greenberg, EP. Bacterial Biofilms: A Common Cause of Persistent Infections. Science 1999, 284, 1318-1322.
3. Rogers, SA.; Huigens, RW.; Cavanagh, J.; Melander, C. Synergistic Effects between Conventional Antibiotics and 2-Aminoimidazole-Derived Antibiofilm Agents. Antimicrobial Agents and Chemotherapy MAY 2010, 54, 2112-2118.
4. Hicks, RP.; Abercrombie, JJ.; Wong, RK.; Leung, KP. Antimicrobial peptides containing unnatural amino acid exhibit potent bactericidal activity against ESKAPE pathogens. Bioorg. Med. Chem. 2013, 21, 205-214.
5. Bard, Alan J.; Faulkner, William F. Electrochemical Methods: Fundamentals and Applications. John Wiley & Sons, Inc.: New York, 2001.

**20.** Motility is crucial for the survival of *Borrelia burgdorferi* in the *Ixodes scapularis* midgut

Syed Sultan, Akarsh Manne and MD Motaleb
Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville.

Motility is crucial for the infectious life cycle of *Borrelia burgdorferi*, the Lyme disease spirochete. We recently reported that the rod-shaped, non-motile, periplasmic flagella-less flaB mutants' burden was significantly reduced in *Ixodes scapularis* ticks. Based on our data, we concluded that spirochetal periplasmic flagella, and consequently motility, are critical not only for optimal survival in ticks, but also for transmission to, and infection of, the mammalian host.

In this study, we directly tested if *B. burgdorferi* motility is specifically involved in the survival in ticks by isolating and then analyzing a paralyzed non-polar mutant Δ*motB*. Specifically, Δ*motB* synthesized wild-type levels of periplasmic flagella as determined by western blot, but was completely non-motile. Using artificial immersion followed by PCR, we found that 100% of the ticks were positive for both wild-type and the mutant spirochetes before feeding on naïve mice. Additionally, using qPCR, wild-type and Δ*motB*-spirochetes load per tick was found to be equivalent, suggesting that spirochetes are able to survive normally in unfed ticks. In contrast, the rate of infection of those ticks by Δ*motB*-spirochetes dropped dramatically from 100% before feeding to 9% after feeding, while this rate remained unchanged for the wild-type or the complemented motB cells. None of those mice fed upon by the mutant spirochete-laden ticks were infected. Based on our tick-mouse studies, we propose a model to describe how motility is likely protecting the spirochetes in the tick midguts. Our model predicts that during or soon after feeding, motility enables *B. burgdorferi* to intimately bind with the midgut epithelial cells by actively swimming in the nutrient rich host blood abundant in the tick midgut. This intimate physical contact between the tick host and the pathogen protects the spirochetes from being killed by harmful molecules present in the midgut.

---

**21.** Vaccinia virus lung infection and the A35R virulence gene

Shayna N. Mooney, Gwendolyn B. Jones, and Rachel L. Roper
Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University

Smallpox killed an estimated 500 million people in the twentieth century alone. Although this fatal infectious disease was eradicated from the world over thirty years ago, it remains an important concern as a bioterrorism agent. Vaccinia virus, the live virus vaccine for smallpox, is extremely dangerous for immunocompromised individuals. Since this cohort comprises a significant portion of the world's population, a safer vaccine is needed. The vaccinia virus A35R gene is highly conserved, and our lab has shown that it increases virulence by inhibiting the body's anti-viral immune responses. When A35R is removed from the virus to create an A35R deletion mutant, the virus becomes attenuated, and immune responses are improved. This study compares the responses of lung leukocyte populations between WR wild type virus infected mice and A35R deletion mutant infected mice to understand the mechanism of A35R immunosuppression. Mice were infected with vaccinia virus (WR and A35RDel), lungs were harvested three days later, and cell populations were quantified using flow cytometric analysis. Initial data showed increased recruitment and/or proliferation of cell populations in response to vaccinia infection, and the A35R gene slightly perturbed populations of B220+ cells (activated B and T cells), macrophages, dendritic cells, and granulocytes. Further repeats of the experiment will be required to look at the kinetics of the immune cell responses and to confirm findings in order to better understand A35R immunosuppression.

---

**22.** Exploration of the mechanism of regulation of motility by Cyclic-di-GMP in *Borrelia burgdorferi*

Syed Z. Sultan and M. A. Motaleb
Department of Microbiology & Immunology, Brody School of Medicine, East Carolina University.

The secondary messenger cyclic-di-GMP is synthesized by diguanylate cyclase enzymes and hydrolyzed by specific phosphodiesterase enzymes containing either EAL or HD-GYP functional domain. The coordinated functions of the synthesizing and degrading enzymes control the level of cyclic-di-GMP which regulates various bacterial activities by binding to an effector molecule called PilZ, RNA Riboswitch or transcriptional regulator such as FleQ. The Lyme disease pathogen *B. burgdorferi* possesses a complete circuit of cyclic-di-GMP regulatory network consisting of a diguanylate cyclase (Rrp1), one EAL domain containing phophodiesterase (PdeA), one HD-GYP domain containing phosphodiesterase (PdeB) and a PilZ domain containing effector protein PlzA. The cyclic-di-GMP regulation system of *B. burgdorferi* is crucial for its tick phase of enzootic life cycle. Additionally, we found that cyclic-di-GMP controls motility in B. burgdorferi. While the wild-type cells run-pause/flex-reverses, the ΔpdeA mutant cells constantly run in one direction with a significantly slower swimming speed. The mechanism of how cyclic-di-GMP controls motility in *B. burgdorferi* is unknown. We propose two possible mechanisms: i) Cyclic-di-GMP directly binds to a flagellar switch protein to regulate motor rotation and speed; or ii) Cyclic-di-GMP binds to a receptor protein which in turn interact with a flagellar switch protein to control the motor function. To demonstrate our hypotheses, we constructed a Δ*rrp1* mutant in the Δ*pdeA* mutant background so that the cells are devoid of cyclic-di-GMP and found that the motility defect of Δ*pdeA* mutant was reverted to wild-type pattern in the *rrp1pdeA* double mutant, indicating that alteration of motility is due to the (increased) level of cyclic-di-GMP. Interestingly, when the receptor protein PlzA was deleted in the Δ*pdeA* mutant background, the motility was not restored to wild-type pattern, suggesting that *B. burgdorferi* may possess additional receptor protein to control motility. In summary, we propose that cyclic-di-GMP controls flagellar rotation either by directly binding to a flagellar protein or by binding to an unknown receptor protein.

---

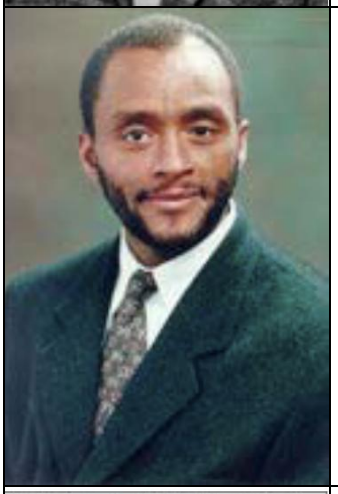
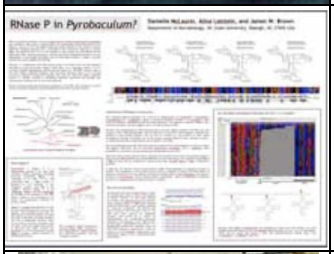
**23.** Isolation and Characterization of Two Genetically Distinct *Paenibacillus larvae* Bacteriophages

Shelby Roland, Kalyani Joshi, William DeFoor, Carlos Goller, Eric S. Miller, and Susan Carson
Biotechnology Program and Department of Microbiology, North Carolina State University, Raleigh, NC 27695

American Foulbrood Disease (AFB) is the most serious bacterial disease affecting honeybees and is caused by the bacterium *Paenibacillus larvae*. The only accepted "treatments" for infected hives in the United States are fumigation and incineration; these are not ideal because both require killing of the colony. While terramycin (oxytetracycline) has been a commonly used preventative, *P. larvae* has developed resistance to this antibiotic. To investigate the diversity and potential applications of bacteriophages that infect *P. larvae*, we collected numerous soil samples from local areas, including soil from two previously diseased apiaries. We used standard enrichment procedures to isolate bacteriophages that infect *P. larvae*. None of these sample collections gave rise to plaques, suggesting that bacteriophage infecting *P. larvae*, and possibly *P. larvae* itself, are not common in soil. We hypothesized that we would find bacteriophage where the host bacterium resides, and thus obtained diseased comb samples from the NC Department of Agriculture. From these samples, two distinct bacteriophages (Lily and Rani) were isolated, each of which shows lytic growth on *P. larvae subspecies larvae* ATCC strain 9545. We report on the host range and morphology of these novel bacteriophages. We also present the preliminary genome annotation of bacteriophage Lily and Rani, as well as genome comparison between these two phages and another newly sequenced P. larvae phage philBB_Pl23. The genome of Lily is 44,952 bases with 73 predicted genes and that of Rani is 38,073 bases with 61 predicted genes. These phages provide an initial view into an under-studied group of bacteriophages that may reveal new biological processes and approaches to controlling AFB.

---

**Presentation Awards**

| | |
|---|---|
|  | **The Mary Poston Award** was established to recognize the best paper given by a student at meetings of the NC Branch of the ASM. Mary Poston was a longtime employee of Duke University who contributed much to the NC Branch and she was held in high esteem both by her colleagues and by medical students. She contributed much to the NC Branch, including service as Branch Secretary-Treasurer from 1950 until her death in 1961. Many letters of appreciation have been written over the years by student recipients of the Mary Poston Award, commenting on the confidence the award gave them and on the importance of the competition for the award as part of their graduate training. |
|  | **The Thoyd Melton Award** was established to recognize an outstanding oral presentation by a Grad student. At the time of his premature death on Nov. 22, 2000, Thoyd Melton was Associate Vice Chancellor for Academic Affairs and Dean of graduate studies at N.C. A&T State University. Prior to this position, Dr. Melton was a member of NC State University's Department of Microbiology and an Associate Dean of the Graduate School. Dr. Melton was very active in research and particularly in graduate education. In 1999, he received the William A. Hinton Research Training Award from ASM. This award honors an individual who has made significant contributions toward fostering the research training of underrepresented minorities in microbiology. |
|  | **The Best Poster award** is open to anyone presenting a poster at the NC ASM meeting. |
|  | **The Paul Phibbs Award** is awarded for the best presentation by an Undergradat NC ASM Branch meetings. |

**A check for $100 will be given for each of these awards at the conclusion of the meeting.**

**Meeting Organization Committee / NC ASM Officers**

| | | |
|---|---|---|
| Eric Anderson | President-elect | East Carolina University |
| Amy Grunden | President | NC State University |
| Sean O'Connell | Past-President | Western Carolina University |
| Marty Roop | Past-Past-President | East Carolina University |
| James W. Brown | Secretary | NC State University |
| Wrennie Edwards | Treasurer | Novartis |
| Ece Karatan | Councilor | Appalachian State University |
| Ed Swords | Alternate Councilor | Wake Forest University |