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KELLY ABSHIRE

Lecturer of Microbiology

Research Interests

My research has focused on investigating the host-pathogen interactions of *Salmonella typhimurium* with macrophage host cells. My primary tool has been two-dimensional gel electrophoresis, which allows examination of the overall pattern of proteins in order to determine which proteins are induced, repressed, and unaffected by a given physiological condition. I have catalogued the cellular response that a certain strain of *S. typhimurium* makes in response to conditions within macrophage host cells. This was done by comparing the proteins induced during intracellular life with those induced in vitro by a variety of stress conditions. The in vitro stress conditions chosen were based on what was known about conditions within macrophage phagolysosomes. This work revealed that at least 36 proteins were induced in the intracellular condition and that induction of several of these proteins may be unique to the intracellular environment. Additional results from these experiments suggested that intracellular *Salmonella* existed in two populations: a few cells were rapidly dividing while a large number remained alive but were not actively growing.

My current goals are to investigate several aspects of pathogen-host interactions. I want to identify a number of the *Salmonella* proteins that are induced in the intracellular condition. It is now possible to cut proteins spots out of 2D gels and have amino acid sequencing done, which allows matching of the unknown proteins to known genes in the sequence databases. Once genes of interest are identified in this way, it should be possible to create mutant strains that could be tested to determine whether the ability to grow within the host cell has been lost. In addition, the work that has been completed concerned just one strain of *Salmonella* and one cell line of macrophages, so it may also be useful to look at a variety of *Salmonella* strains in combination with macrophages from different sources. I am also planning to work with Dr. Joseph Carlin in using the 2D gel approach to investigate the interaction of the obligate intracellular pathogen, *Chlamydia trachomatis*, with host cells. I hope to be able to develop a catalogue of proteins important for this pathogen's virulence.

Research Interests

The ability of bacteria to cause diseases depends on the expression of a large number of factors. Some of these factors are essential for pathogenic bacteria to attach, invade, and obtain essential nutrients from the host. Iron is one of these essential nutrients and almost all-living cells require it for cell metabolism and multiplication. Iron is also a very important environmental signal to bacteria for coordinated regulation of gene expression. Most of the iron in nature and body fluids is highly insoluble and is very toxic, causing severe damage to DNA and lipids. Bacteria have evolved different mechanisms to acquire this metal and store it as nonharmful complexes inside the cells. Production of low molecular-weight chelating compounds (siderophores) and direct binding of iron-chelating proteins (transferrin and lactoferrin) are among the most important bacterial systems involved in iron acquisition, and they represent important virulence factors. Once the iron is inside the bacterial cells, it is complexed to iron-binding proteins, some of them similar to eukaryotic ferritins, thus preventing the generation of highly toxic free radicals.

Current Projects

Our laboratory is currently analyzing the siderophore-mediated iron uptake mechanism of different strains of *Acinetobacter*, an unexplored aspect of a bacterial species that is widely distributed throughout the environment. We have characterized a novel iron uptake system in a human isolate of *A. baumannii* that includes a catechol siderophore and iron-regulated membrane proteins that transport the iron-siderophore complexes from the extracellular medium into the bacterial cytosol. Furthermore, we have reason to believe that this bacterium expresses more than one iron uptake system and may use siderophores secreted by other bacterial species that include a fish pathogen that causes disseminated infections in salmonids. In addition, we have initiated the molecular and genetic analysis of iron acquisition in *Haemophilus influenzae* biogroup aegyptius, which causes fatal infections in children, and *Actinobacillus actinomycetemcomitans*, a bacterium that is involved in the infection and destruction of dental tissues. These two bacterial species obtain iron either by direct interaction with transferrin or the expression of novel membrane transport systems that do not require a ligand or a siderophore molecule.

Our research goals also include the identification and characterization of novel bacterial genes involved in the pathogenesis of infectious diseases. We have recently identified pieces of DNA present only in the genome of invasive strains of *H. influenzae* biogroup aegyptius, which causes the destruction of the vascular system in children. These DNA pieces were isolated and cloned using a novel technical approach and preliminary analysis indicates that they contain unique genes that do not share homology with already identified genes deposited in the GenBank database.

These results indicate that the virulence of this pathogen is most likely due to genetic determinants and bacterial products that remain to be characterized.

To achieve these objectives we use classical bacterial genetic methods together with modern molecular biology techniques such as the construction of gene libraries using chromosomal DNA and cosmid cloning vectors, DNA nucleotide sequencing, and characterization of gene products such as messenger RNAs and proteins. The regulation of the expression of these genes is analyzed with northern blots, RNase protection assays, and reverse transcription-polymerase chain reaction (RT-PCR) experiments. Transposition and site-directed mutagenesis combined with genetic complementation experiments are used to test the biological role of the products encoded by putative bacterial virulence genes. In addition, genes encoding proteins involved in either iron acquisition or host cell invasion and destruction are cloned in expression vectors that allow the chemical and immunological characterization of these proteins by techniques such as gel electrophoresis, immunoblotting and chemical cross linking.

Selected Publications

Tomaras, A. P., C. W. Dorsey, R. E. Edelman, and **L. A. Actis**. Attachment to and biofilm formation on abiotic surfaces by *Acinetobacter baumannii*: involvement of a novel chaperone-usher pili assembly system. *Microbiology*, 149, 3461-3484, 2003.

Dorsey, C. W. Dorsey, A. P. Tomaras, P. L. Connerly, M. E. Tolmasky, J. H. Crosa, and **L. A. Actis**. The siderophore-mediated iron acquisition systems of *Acinetobacter baumannii* ATCC 19606 and *Vibrio anguillarum* 775 are structurally and functionally related. *Microbiology*, 150, 3657-3667, 2004.

McGillivray, G., A. P. Tomaras, E. R. Rhodes, and **L. A. Actis**. Cloning and sequencing of a genomic island found in the Brazilian purpuric fever clone of *Haemophilus influenzae* biogroup aegyptius. *Infection and Immunity*, 73, 1927-1938, 2005.

Rhodes, E. R., A. P. Tomaras, G. McGillivray, P. L. Connerly, and **L. A. Actis**. Genetic and functional analysis of the *Actinobacillus actinomycetemcomitans* AfeABCD siderophore independent iron-acquisition system. *Infection and Immunity*, 73, 3658-3763, 2005.

McGillivray, G., L. M. Smoot, and **L. A. Actis**. Characterization of the IgA1 protease from the Brazilian purpuric fever strain F3031 of *Haemophilus influenzae* biogroup aegyptius. *FEMS Microbiology Letters*, 250, 229-236, 2005.

MITCHELL F. BALISH

Assistant Professor of Microbiology

Research Interests

The cytoskeleton is a set of dynamic subcellular structures that imparts upon cells numerous abilities that are essential to life processes: 1) to maintain and modulate shape; 2) to divide; 3) to move; 4) to transport molecules from one part of the cell to another; and 5) to organize and localize molecules and structures within the cell. Study of the cytoskeleton has been carried out for many years in eukaryotic cells. However, for many years it was thought that prokaryotic cells neither required nor possessed cytoskeletons, as the cell wall provided both morphological stability and flexibility, the small size of the cells obviated the need for long-distance transport and subcellular organization, and division and non-flagellum-based movement were carried out by mysterious, specialized structures.

More recently, however, the accepted view of the inner workings of the bacterial cell has irreversibly changed on account of two realizations. First, dynamic structures that are similar to eukaryotic cytoskeletal components have been identified and characterized in many bacteria; these structures have turned out to be especially important with regard to cell shape, cell division, and subcellular organization. Second, appreciation of bacterial diversity has increased, revealing species that differ considerably from model bacteria. Among these are those bacteria of the class *Mollicutes*, which are the smallest organisms capable of being grown in pure culture.

Mollicutes, which lack cell walls, have small genomes, and are generally deficient in biosynthetic pathways, are evolutionarily related to the low G+C Gram-positive bacteria. In nature they are associated with animal and plant hosts. The best-studied *Mollicutes* are those of the genus *Mycoplasma* (trivial name mycoplasmas), which infect vertebrate hosts, including humans. Of these, the best-characterized is the human pathogen *Mycoplasma pneumoniae*, which is a leading cause of tracheobronchitis and atypical ("walking") pneumonia, especially in children and young adults. Related species like *Mycoplasma genitalium*, *Mycoplasma penetrans*, and the newly-described *Mycoplasma amphoriforme* also infect humans, and other mycoplasmas like *Mycoplasma gallisepticum* are significant livestock pathogens.

Many mycoplasmas, including all these species, have prominent polar protrusions called attachment organelles. These structures, which are constructed from cytoskeletal proteins found only among certain mycoplasmas, are essential for effective colonization of host tissue and virulence as well as for gliding motility of mycoplasma cells along surfaces, a process whose physiological role

might also be related to host interactions. How the proteins of the attachment organelle specifically function in its architecture and virulence-related properties is largely unknown.

In addition to its roles in adherence to host cells and gliding motility, the *M. pneumoniae* attachment organelle is intricately involved in the process of cell division. Remarkably, in apparent coordination with the onset of DNA replication, the attachment organelle duplicates, and one of the two attachment organelles migrates from one pole of the cell to the other. Following division, the process begins again.

The molecular basis for both gliding motility and attachment organelle duplication/migration is almost entirely unknown, not only in *M. pneumoniae* but also in other mycoplasmas, many of which, including *M. penetrans*, lack homologs of the known attachment organelle proteins. The focus of the research in our laboratory is elucidating the molecular underpinnings of attachment organelle morphology, cell division, and gliding motility in a variety of mycoplasma species. This is carried out through techniques that include real-time microcinematographic imaging, fluorescence and electron microscopy, molecular biology, and protein biochemistry.

Selected Publications

May, M., G.J. Ortiz, L.D. Wendland, D.S. Rotstein, R.F. Relich, **M.F. Balish**, and D.R. Brown. *Mycoplasma insons* sp. nov., a twisted mycoplasma from green iguanas (*Iguana iguana*). FEMS Microbiol. Lett. In press.

Jordan, J.L., H.-Y. Chang, **M.F. Balish**, L.S. Holt, S.R. Bose, B.M. Hasselbring, R.H. Waldo III, T.M. Krunkosky, and D.C. Krause. 2007. Protein P200 is dispensable for *Mycoplasma pneumoniae* hemadsorption but not gliding motility or colonization of differentiated bronchial epithelium. Infect. Immun. **75**:518-522.

Balish, M.F., and D.C. Krause. 2006. Mycoplasmas: a distinct cytoskeleton for wall-less bacteria. J. Mol. Microbiol. Biotechnol. **11**:244-255.

Hatchel, J.M., R.S. Balish, M.L. Duley, and **M.F. Balish**. 2006. Ultrastructure and gliding motility of *Mycoplasma amphoriforme*, a possible human pathogen. Microbiology **152**:2181-2189.

Balish, M.F. 2006. Subcellular structures of mycoplasmas. Front. Biosci. **11**:2017-2027.

ANNETTE BOLLMANN

Assistant Professor of Microbiology

Research Interests:

My research interests are the isolation, ecology, and physiology of difficult to culture bacteria with special emphasis on bacteria involved in environmentally important processes

Culturing the unculturables: Approximately 99% of the bacteria known by molecular markers have never been cultured in the laboratory. These bacteria exhibit a large source of unknown secondary metabolites and genes involved in bioremediation and other biotechnological interesting processes. A combination of in situ (diffusion chambers) and in vitro methods has been used to cultivate previously uncultured bacteria. These methods have been successfully applied to different environments, like freshwater sediment, contaminated surface sediments and wastewater. Clear differences between the isolates obtained with and without passage of the material through the diffusion chamber were discovered, indicating that the diffusion chamber is a good additional tool to enrich and isolate new bacteria [several publications in prep].

Future projects:

- Isolation of bacteria using a combination of in situ and in vitro methods from different environments
- Characterization of new and interesting isolates

Pure cultures versus consortia: In nature bacteria populations occur in communities and consortia consisting of assembly's of two (and more) different microorganisms. The bacteria in these consortia interact and perform reactions that no one of the partners can perform alone. Beneficial interactions include commensalism, cross feeding, co metabolism, and signaling. Due to all these interactions microbial consortia can be seen as highly sophisticated units.

Future projects:

- Consortia in bioremediation
- Consortia in general and patterns behind positive inter-actions among bacteria

Ammonia-oxidizing bacteria (AOB) are chemolitho-autotrophic bacteria gaining their energy by oxidizing ammonia to nitrite. Ammonia oxidation is the first and very often rate-limiting step in the nitrification process. AOBs can be found in very different environments, like soils, marine and freshwater columns and sediments, and wastewater. My research is focused on AOBs adapted to low ammonium concentrations. These bacteria were enriched in chemostats and used to investigate their competition and starvation behavior, as well as their interactions with heterotrophs [3; 4; in prep].

Future projects:

- Whole genome sequence of the new isolate *Nitrosomonas* sp Is79A3
- Further investigations of the physiology of AOBs adapted to low ammonium concentrations

Selected Publications:

Bollmann, A. & R. Conrad. 1998. Influence of O₂ availability on NO and N₂O release by nitrification and denitrification in soils. *Global Change Biology* 4: 387-396

Bollmann, A. & H.J. Laanbroek. 2001. Continuous culture enrichments of ammonia-oxidizing bacteria at low ammonium concentrations. *FEMS Microbiology Ecology* 37: 211-221.

Bollmann, A. & H.J. Laanbroek. 2002. Influence of oxygen partial pressure and salinity on the community composition of the ammonia-oxidizing bacteria in the Schelde estuary. *Aquatic Microbial Ecology* 28: 239-247.

Bollmann, A., M.-J. Bär-Gilissen, H.J. Laanbroek. 2002. Growth at low ammonium concentrations and starvation response as potential factors involved in niche differentiation among ammonia-oxidizing bacteria. *Applied and Environmental Microbiology* 68: 4751-4757.

Bollmann, A., I. Schmidt, A.M. Saunders, & M.H. Nicolaisen. 2005. Influence of starvation on the potential ammonia-oxidizing activity and *amoA* mRNA of *Nitrospira briensis*. *Applied and Environmental Microbiology* 71: 1276-1282.

Bollmann, A., K. Lewis, & S. Epstein. 2007. Incubation of environmental samples in a diffusion chamber increases the diversity of recovered isolates. *Applied and Environmental Microbiology* 73:6386-6390.

Research Interests

Adenovirus (Ad) is a linear double-stranded DNA virus. Successful lytic infection by Ad requires a productive interaction with its host cell, creating an environment that is conducive to efficient viral gene expression, replication, and assembly of new progeny virus. Ad regulatory proteins derived from early region 4 (E4), E4-11K and E4-34K, and an early region 1b protein, E1b-55K, are important in cell transformation, viral replication, and late gene expression; they are also able to interact with host double-strand break repair (DSBR) proteins and affect their localization and stability. E4 mutants have their genomes "repaired" in infected cells to form concatemers of several genome lengths. My group is investigating the role of host DSBR processes in the replication and late gene expression defects displayed by E4 mutants lacking E4-11K and E4-34K. We are studying the relationship between the function of E4 proteins in inactivating DSBR, and their role in promoting viral replication and gene expression. We are investigating the role of genome concatenation for viral DNA replication and late gene expression in E4 mutant infections. We are also studying the interactions of host DSBR proteins with viral replication foci and viral DNA in E4 mutant infections. These studies are expected to further our understanding of how viral genes affect host cell activities, and how they may ultimately affect cell growth and transformation as they reprogram the machinery of the host cell to promote a successful lytic viral infection.

Selected Publications

- Mathew, S.S. and **E. Bridge**. 2007. The cellular Mre11 protein interferes with adenovirus E4 mutant DNA replication. *Viol.* **365**:346-55
- Jayaram, S., and **E. Bridge**. 2005. Genome concatenation contributes to the late gene expression defect of an adenovirus E4 mutant. *Viol.* **342**:286-296.
- Bridge, E.**, K. Mattsson, A. Aspegren, and A. Sengupta. 2003. Adenovirus early region 4 promotes the localization of splicing factors and viral RNA in late phase interchromatin granule clusters. *Viol.* **311**:40-50.
- Corbin-Lickfett, K., and **E. Bridge**. Adenovirus E4-34kDa requires active proteasomes to promote late gene expression. *Viol.* **315**:234-244.
- Carter, C.C., R. Izadpanah, and **E. Bridge**. 2003. Evaluating the role of CRM1-mediated export for adenovirus gene expression. *Viol.* **315**:224-234.
- Aspegren, A., and **E. Bridge**. 2002. Release of snRNP and RNA from transcription sites in adenovirus-infected cells. *Exp. Cell Res.* **276**:273-283.

Research Interests

Chlamydia trachomatis is a major sexually transmitted infection in the United States, with an estimated 3-5 million new cases per year. Furthermore, infections in 80% of women and 50% of men produce little to no symptoms, and thereby remain undetected and untreated. Unfortunately, untreated infection can lead to tubal infertility and ectopic pregnancy. Although these infections can be detected by screening sexually active individuals, widespread screening is not routinely performed. An effective alternative would be the development of a vaccine. However, traditional approaches to vaccine development have been unsuccessful, simply because we do not yet understand the immunobiology of *Chlamydia*. My research has been focused on gaining that understanding, such that we might someday be able to prevent chlamydial infection. Chlamydial infection triggers the production of a variety of immunomodulatory molecules, collectively known as cytokines. Interferon-gamma, a cytokine produced in response to infection, induces several antimicrobial mechanisms, including the breakdown of tryptophan by indoleamine dioxygenase (IDO). Activation of IDO rapidly depletes intracellular tryptophan, thereby starving tryptophan-dependent *Chlamydia* of an amino acid it requires for protein synthesis. While this works effectively in tissue culture, the fact that chlamydial infections are widespread and often asymptomatic suggests that *Chlamydia* are somehow evading this immunological defense mechanism.

Current Projects

Currently, the goal of my laboratory is to determine how *Chlamydia* evades destruction by IDO. Although IFN- γ induces IDO activity, cytokines involved in inflammation, IL-1 and TNF- α , can enhance the cell's response to interferon. To understand the nature of this interaction, we have been analyzing changes in IDO gene expression using cells that we transfected with a green fluorescent protein (GFP) gene under the control of the IDO regulatory DNA sequence. Our results indicate that regulation of IDO is multifactorial; three different transcription factors contribute to IDO regulation. Although signal transducer and activator of transcription-1 (STAT-1) is required for activation by interferon, activation of nuclear factor- κ B (NF- κ B) by pro-inflammatory cytokines is required for synergistic expression of a third key transcription factor, interferon regulatory factor-1 (IRF-1), which is required for full activation of the IDO gene. By understanding the nature of the IDO gene regulation, we have established a framework for identifying potential effects of chlamydial infection on these pathways. In addition, we have shown that these cytokines up-regulate the expression of pro-inflammatory cytokine receptors, and that this increase in receptor expression renders the cells more sensitive to cytokine treatment. These experiments, combined with

our knowledge of IDO gene regulation, has painted a clearer picture of the complexity of the interaction between multiple cytokines and the generation of a protective response to *Chlamydia*. Another goal has been to assess the effect of intracellular infection on the ability of the infected cell to respond to cytokine signals. The use of 2-color flow cytometry permits the assessment of immune responsiveness in individual infected cells. Using this approach, we have discovered that the engagement of toll-like receptors 2 and 4 by *Chlamydia* up-regulates receptors of interferon-gamma and other pro-inflammatory cytokines by more than 10-fold. This was unexpected, inasmuch as increased receptor expression should lead to greater IDO activity. However, these cells do not gain increased responsiveness to the respective cytokines. Rather, the signaling pathway used by these receptors appears impaired. Discovering the nature of this impairment is currently underway.

Selected Publications

- Shirey, K A., J.-Y. Jung, and **J.M. Carlin**. 2006. Up-regulation of interferon- γ receptor expression due to *Chlamydia* Toll-like receptor interaction does not enhance STAT-1 signaling. *Infect. Immun.* 74:6877-6884.
- Robinson, C.M., P.T. Hale, and **J.M. Carlin**. 2006. NF- κ B contributes to indoleamine dioxygenase transcriptional synergy by IFN- γ and tumor necrosis factor- α . *Cytokine* 35:53-61.
- Shirey, K A., and **J.M. Carlin**. 2006. Chlamydiae modulation of IFN γ -, IL-1 β -, and TNF α -receptor expression in HeLa cells. *Infect Immun.* 74:2482-2486.
- Shirey, K A., J.-Y. Jung, G.S. Maeder, and **J.M. Carlin**. 2006. Upregulation of IFN- γ receptor expression by proinflammatory cytokines influences IDO activation in epithelial cells. *J. Interferon Cytokine Res.* 26:53-62.
- Robinson, C.M., P.T. Hale, and **J.M. Carlin**. 2005. The role of IFN- γ and TNF- α responsive regulatory elements in the synergistic induction of indoleamine dioxygenase. *J. Interferon Cytokine Res.* 25:20-30.
- Robinson, C.M., K A. Shirey, and **J.M. Carlin**. 2003. Synergistic transcriptional activation of indoleamine dioxygenase by interferon-gamma and tumor necrosis factor-alpha. *J. Interferon Cytokine Res.* 23:413-421.
- Currier, A.R., M.H. Ziegler, M.R. Riley, T.A. Babcock, V.P. Telbis, and **J.M. Carlin**. 2000. Tumor necrosis factor- α and LPS enhanced interferon-induced antichlamydial indoleamine dioxygenase activity independently. *Cytokine* 12:588-594.

Research Interests

My research interests encompass the basic and applied areas of invertebrate viruses, particularly baculoviruses and ascoviruses. Baculoviruses have long been used as insect biological control agents in agriculture and forestry. However, they are slow in action and there is a need for improvement. One of my research areas is the engineering of baculoviruses to enhance their efficacy. For instance, by using a novel fusion protein strategy, the insecticidal protein could be delivered to the midgut of the insect to cause early mortality. We have provided strong evidence that fusing insecticidal protein or reporter protein green fluorescent protein (GFP) to the C-terminus of polyhedrin of *Autographa californica* nucleopolyhedrovirus (AcMNPV) could result in the fusion protein incorporated in the occlusion bodies (polyhedra) of baculoviruses. In addition, this strategy could be applied to other basic research projects such as protein-protein interaction, protein polymerization and viral occlusion. For example, we could use different regions of the polyhedrin to fuse with GFP to eventually pinpoint the amino acid(s) involved in the polyhedrin intermolecular interaction. Information from this research will elucidate the dynamics in the polyhedrin crystallization. It may provide ideas for other protein research. Besides, we have also found that some NPVs produce occlusion bodies (polyhedrin) without virions embedded both naturally or in vitro. As NPVs need occlusion bodies to perpetuate in the natural environment, and need virions in the occlusion bodies to initiate replication in the hosts. It is important to find out how this occurs. It is hypothesized that some virion capsid protein(s) interact with the polyhedrin protein. We are in the process of isolating the mutant that lacks occlusion. By functional genomic study, we are probably able to map the region in the genome to find the mutation. The yeast two-hybrid system will be used to support the protein-protein interaction and understand how virions are occluded in the late phase of viral replication.

Another group of viruses that I am interested in working are ascoviruses of insects. As these ascoviruses are quite new to the research community of virology and not much information is yet available. Transfection of viral DNA to cells in vitro was not successful, however, transfection of viral DNA of ascovirus into larvae has been developed. This technique will be used to study genetics of ascoviruses as to how they replicate in the cells. Ascovirus genomic libraries have been constructed and are used to analyze genes involved in early stages of viral replication by mRNA studies using the Northern hybridization method. Ascoviruses have a unique feature in infecting cells by cleaving the cells to form compartments or vesicles. By gene knockout and functional studies, this unique feature may be elucidated.

Selected Publications

- Wang, L.-H, T. Z. Salem, D. J. Campbell, C. M. Turney, C. M. S. Kumar and **X.-W. Cheng** (2009). Characterization of a virion occlusion defective *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) mutant lacking the p26, p10 and p74 genes. *Journal of General Virology* **90**: 1641-1648. (Figures on cover page).
- Cheng, X.-W.** and D.-E. Lynn. (2009). Baculovirus interactions in vitro and in vivo. In *Advances in Applied Microbiology* Volume 68 pp. 218-239. Edited by A. I. Laskin, G. M. Gadd, and S. Sariaslani. Elsevier Inc. San Diego, California. (invited book chapter)
- Cheng, X.-W.**, G.R. Carner, M. Lange, J.A. Jehle, and B.M. Arif. 2005. Biological and Molecular Characterization of a Multicapsid Nucleopolyhedrovirus from *Thysanoplusia orichalcea* (L.) (Lepidoptera: Noctuidae). *Journal of Invertebrate Pathology*. 88:126-135.
- Cheng, X.-W.**, T.R. Henriques, S.R. Coppens, Q. Feng, A. Retnakaran, P.J. Krell, and B.M. Arif. 2003. Strategy to screen long DNA inserts in *E. coli*. *BioTechniques* 34, (6).
- Zheng, Y., S. Zheng, **X.-W. Cheng**, E.J. Lingohr, T. Ladd, P.J. Krell, B.M. Arif, A. Retnakaran and Q. Feng. 2002. A molt-associated chitinase cDNA from the spruce budworm *Choristoneura fumiferana*. *Insect Biochem. and Mol. Biol.* **32**:1813-1823.
- Cheng, X.-W.**, P. J. Krell and B.M. Arif. 2001. P34.8 (GP37) is not essential for baculovirus replication. *J. Gen. Virol.* **82** (2), 299-305.
- Cheng, X.-W.** and G.R. Carner. 2000. Characterization of a single-nucleocapsid nucleopolyhedrovirus of *Thysanoplusia orichalcea* L. (Lepidoptera:Noctuidae) from Indonesia. *J. Invert. Pathol.* **75**:279-287.
- Cheng, X.-W.** G.R. Carner and B.M. Arif. 2000. A new ascovirus from *Spodoptera exigua* and its relatedness to the isolate from *Spodoptera frugiperda*. *J. Gen. Virol.* **81**: 3083-3092.

MARJORIE M. (KELLY) COWAN

Regional Dean, Middletown Campus

Professor of Microbiology

Research Interests

My research program follows three strands. The **first** is the detailed physicochemical description of bacterial adsorption to mammalian host surfaces, and its inhibition. The ultimate goal of this research is the prevention of infection by the blocking of bacterial binding. Bacteria colonize a wide range of epithelial cells in order to begin their infections – cells in the oral cavity, the pharynx, the gastrointestinal tract, the urinary and reproductive tracts. All of these epithelial cells possess different carbohydrates on their surfaces. Bacteria have evolved proteins that match the specific types of sugars on the cells in their appropriate niche. Many researchers focus on the chemistry of individual interactions in order to prevent them, as we do. In addition, however, our lab recognizes a further critical component of bacterial adsorption – which can be called the physical chemistry of the bacterium's approach to its specific receptor.

Bacterial cells, in addition to being living organisms, are physical particles, and must obey physical laws of attraction and repulsion. Often the physical forces governing a cell's approach to epithelium are initially more important than the actual lock-and-key chemistry that will finally secure a bacterium firmly to host tissue. The predominant forces are van der Waals, electrostatic and hydrophobic interactions. Overlooking these forces leads to ambiguities, apparent in the literature, when attempting to identify specific lock-and-key chemical pairs on bacteria and host cells. Currently our lab is engaged in a physicochemical description of the adhesion of *Streptococcus pyogenes* to pharyngeal epithelium. Our approach uses a kinetic analysis of the interaction of bacteria and epithelial cells. Rate constants for adsorption and desorption indicate whether there are two distinct phases of adhesion. They provide a quantitative method for assessing the relative importance of various bacterial macromolecules in the distinct phases and the effects of adhesion-inhibitory treatments.

Our examinations of the physicochemical aspects of the adhesion process are facilitated by a flow cytometric kinetic method developed in our laboratory, which overcomes many of the problems associated with *in vitro* analyses of adhesion.

The **second** strand of research concerns a novel inhibitory strategy that our laboratory has developed. We used a bioinformatics analysis of the 3D architecture of adhesion binding sites to discover that although bacterial adhesion proteins are very diverse, they have certain "consensus" residues in contact with the carbohydrate ligand. In other words, the binding sites from many different bacteria (and viruses, protozoa and fungi) very often rely on a consensus 3D motif to effect good chemical fits with sugar receptors. This finding guides our current research in using enzymes that cripple the consensus amino acids

as a broadly effective means of inhibiting bacterial attachment. Some of the model infection systems we examine are the Type 1- and P-fimbriated *Escherichia coli*, pathogenic in the urinary tract, *Streptococcus sobrinus*, pathogenic in the oral cavity, and *Streptococcus pneumoniae*, responsible for many infections in immunocompromised patients as well as a large percentage of cases of otitis media (ear infections).

Third, our lab is combining an appreciation of the role of adhesion in the initiation of infection with a search for novel antimicrobials. Currently, massive screening of plants is being performed by laboratories and industries world-wide in an attempt to find new chemical compounds that can be used as antibiotic-like substances. The ethnobotanical methodology involves an initial screening of plant extracts to detect their ability to kill or inhibit the growth of disease-causing microorganisms. Our belief is that substances that prevent adhesion of microorganisms will also be effective anti-microbials. Our laboratory hopes to add to this research and recognition that plant compounds that act in this way (anti-adhesive) are being missed in the screening procedures currently used. The research detailed above has shown that particular enzymes are capable of disrupting consensus binding-site sequences. Some of these enzymes are common and plentiful in plant tissues. In addition plants possess many other peptides and phenolic derivatives which should be tested for their anti-colonization effects. We are investigating plants known to be effective against infection (used effectively by native cultures to treat infections), yet have minimal microbial killing or growth-inhibitory activity. We recently identified an adhesion inhibitory compound in *Solanum tuberosum* (potato). We are also beginning to investigate the link between our results and the fact that many cultures use potato peelings to assist in burn and wound healing.

Selected Publications

C.E. Budu, J. Luengpailin, **M.M. Cowan** and R.J. Doyle. 2003. Virulence Factors of *Porphyromonas gingivalis* are Modified by Polyphenol Oxidase and Asparaginase. *Oral Microbiol. Immunol.* **18**:313-317.

Cowan, M.M., K. Abshire, S.L. Houk, S.N. Evans. 2003. Antimicrobial efficacy of a silver-zeolite matrix coating on stainless steel. *J. Industrial Microbiol. Biotechnol.* **30**:102-106.

Sethman, C.R., R.J. Doyle, and **M.M. Cowan**. 2002. Flow cytometric evaluation of *Streptococcus pyogenes* adhesion to epithelial cells. *J. Microbiol. Methods* **51**:35-42.

Cowan, M.M., S. Leungpailin, E.A. Horst, and R.J. Doyle. 2000. The effect of plant polyphenol oxidase on colonization factors of *Streptococcus sanguis* 6715. *Antimicrobiol. Agents Chemother.* **44**:2578-2580.

Research Interests

Methanosarcina acetivorans is a methylotrophic methanogenic archaeon capable of growth in many environments and on a wide variety of substrates. Research on this and related organisms has revealed the existence of analogous pathways of methanol- and methylamine-dependent methanogenesis involving homologous corrinoid binding proteins and non-homologous methanol and methylamine methyltransferases. In close proximity to the genes encoding the methylamine specific methyltransferases and corrinoid proteins there have been genes encoding putative permeases: *mtmP*, *mtbP*, and *mttP*. The precise role of these putative permease genes has never been demonstrated. One of the goals of my laboratory is to demonstrate the role of these genes in methylamine dependent methanogenesis by generating knock-out mutants in *M. acetivorans* and by functional expression of the genes in *E. coli*.

The genes encoding the methylamine methyltransferases MttB, MtbB, and MtmB all contain an in-frame amber UAG codon. This UAG codon encodes the 22nd genetically encoded amino acid pyrrolysine. The genes necessary for the translation of the UAG codon are found within the *pylTSBCD* operon in *Methanosarcina barkeri* as well as other methylotrophic methanogens. Thus far, only limited evidence of horizontal gene transfer of the *pyl* operon exists, with the primary example being a Gram positive bacterium *Desulfitobacterium hafniense*, however the possibility remains that other organisms have obtained this gene cluster as well. Another goal of my laboratory will be to screen difficult to culture environmental isolates obtained in collaboration with Dr. Annette Bollmann which may be carrying the *pyl* operon.

GARY R. JANSSEN
Associate Professor of Microbiology

Research Interests

Research in our laboratory is focused in three areas: 1) translation of leaderless mRNA in bacteria, 2) analysis of antibiotic resistance genes, and 3) regulatory effects of phosphate on primary and secondary metabolism in *Streptomyces*.

The majority of prokaryotic mRNAs contain an untranslated leader region, located 5' to the coding sequence, that is believed to function in the association of mRNA that naturally lack upstream leader sequences but continue to be translated. Among the questions we want to ask are the following: What are the nucleotide or structural features that determine the translational efficiency of leaderless mRNA? Do leaderless mRNAs translated by all ribosomes or by a specialized subpopulation of ribosomes? Is the translation of leaderless mRNA influenced by physiological conditions? In addition, we have demonstrated that conventionally leadered mRNA continue to be translated after removal of their upstream leader regions; efforts are under way to identify possible advantages of the leadered or unleadered state for mRNA.

In antibiotic-producing streptomycetes, the antibiotic resistance genes are physically linked to antibiotic production genes. In order to prevent suicide, expression of resistance must precede antibiotic production. We are using a molecular biological approach to the study of resistance gene regulation and the relationship between expression of resistance gene(s) and antibiotic production.

Antibiotic production and secondary metabolism in *Streptomyces* are regulated, in part, by the extracellular levels of phosphate. We have isolated mutants that are impaired in the normal regulation of antibiotic production by phosphate. These mutations are being mapped to the *Streptomyces* chromosome and a gene bank of *Streptomyces* DNA is being used to isolate the genes involved in phosphate regulation of primary and secondary metabolism.

Selected Publications

O'Donnell, S. and **G.R. Janssen**. 2002. Leaderless mRNAs bind 70S ribosomes more strongly than 30S subunits in *Escherichia coli*. *J. Bacteriol.* **184**:6730-6733.

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Martin-Farmer, J. and **G.R. Janssen**. 1999. A downstream CA repeat sequence increases translation from leadered and unleadered mRNA in *Escherichia coli*. *Mol. Microbiol.* **31**:1025-1038.

Van Etten, W. and **G.R. Janssen**. 1998. An AUG initiation codon, not codon-anticodon complementarity, is required for the translation of unleadered mRNA in *Escherichia coli*. *Mol. Microbiol.* **27**:987-1001.

Wu, C.J. and **G.R. Janssen**. 1997. Expression of a streptomycete leaderless mRNA encoding chloram-phenicol acetyltransferase in *Escherichia coli*. *J. Bacteriol.* **179**:6824-6830.

Research Interests

We are studying ice nucleating active (INA) fungi and bacteria. Small volumes of pure water remain liquid, i.e. do not freeze, when cooled to temperatures as low as approximately -40°C unless a nucleating substance is present. The most efficient and most common known nucleators, found in leaves, in clouds, oceans, and animals, are INA bacteria and fungi. These microbes can regulate the freeze-susceptibility of insects, most of which are freeze-intolerant. In the winter many of these insects survive by increasing their capacity to supercool, and thus, avoid the lethal effects of internal ice formation.

Increases in supercooling capacity require the removal or inactivation of heterogeneous ice-nucleating catalysts. Although several lines of evidence suggest that ice nucleation begins in the gut, the precise nature of the ice nucleating agent regulating supercooling in freeze-intolerant insects is not clearly established. In collaboration with zoology, our laboratories provided the first evidence that the ingestion or cuticular application of INA bacteria significantly decreases the supercooling capacity and, as a result, reduces the cold tolerance of a wide variety of insects. We also established that INA bacteria are normal flora in the gut of insects. The selected insect host for our research, the Colorado potato beetle, is freeze-intolerant, and is the primary potato pest in North America, appearing to be an excellent candidate for control using INA microorganisms. Ultimately, we hope to develop probes to detect the presence of INA genotypes in and on insects to accompany cultural fluorescent and nucleation studies which detect the presence of the INA positive phenotype. We seek to determine the potential for use of INA bacteria and fungi as biological control agents for insect pests, initially the Colorado potato beetle.

Furthermore, we are exploring differential effects of antifungal agents upon conidial and hyphal morphologic forms of the increasingly frequent fungal opportunists *Aspergillus fumigatus*, *Fusarium oxysporum* and *Candida albicans*. Our goal is to identify antifungal agents, including natural antifungal compounds, which inhibit growth of the *in vivo* fungal hyphal forms and/or inhibit germination of infective fungal conidia, an initial step occurring prior to fungal invasion of host tissue. Our experiments utilize classical microtiter and cultural methods together with flow cytometry and fluorescent microscopy.

Selected Publications

Humphreys, T.L., L.A. Castrillo, and **M.R. Lee**. 2001. Sensitivity of partially purified ice nucleation activity of *Fusarium acuminatum* SRSF 615. *Current Microbiology* **42**:330-338.

Castrillo, L.A., R.E. Lee, J.A. Wyman, **M.R. Lee**, and S.T. Rutherford. 2001. Field persistence of ice-nucleating bacteria in overwintering Colorado potato beetles. *Biological Control* **21**:11-18.

Castrillo, L.A., R.E. Lee, **M.R. Lee** and J.A. Wyman. 2000. Long-term retention of ice-nucleating active *Pseudomonas fluorescens* by overwintering Colorado potato beetles. *CryoLetters* **21**:5-12.

Lee, M.R., R.E. Lee, J.M. Strong, S. R. Minges, and J.A. Mugnano. 1998. Reduction of insect cold-hardiness using ice-nucleating active fungi and surfactants. *Entomologia Experimentalis et Applicata* **89**:103-109.

RACHAEL MORGAN-KISS

Assistant Professor of Microbiology

Research Interests

My main research interests focus on adaptation of microorganisms to their environments, in particular extremophilic photosynthetic lifeforms. In an attempt to understand how an organism has adapted to fill its unique niche, my research programme has an overarching goal to pursue questions that involve both natural populations of extremophilic photoautotrophs as well as isolated microbes grown under controlled laboratory conditions. I am pursuing a number of ongoing and new projects that encompass this goal.

One extreme environment which is studied in my laboratory is permanently cold ecosystems. More than 70% of the world exists as cold ecosystems that have stable temperatures below or close to the freezing point of water. Photoautotrophic fixation of carbon dioxide into dissolved carbon drives microbial productivity in most low temperature aquatic ecosystems. Despite their global significance, the psychrophiles, and in particular cold adapted photoautotrophs, are poorly understood.

Current and Future Projects

In an effort to understand photosynthetic adaptation to persistently low temperature environments, I work on the physiology and biochemistry of the photosynthetic apparatus of two psychrophilic green algae, *Chlamydomonas raudensis*, and a newly discovered *Chlorella* sp. *C. raudensis* was isolated from a permanently ice-covered aquatic environment in the Dry Valleys of Antarctica, where it is adapted to low temperatures, extreme shade, high salinity and year-round stable environmental conditions. Over a decade of work has made this enigmatic alga one of the most well characterized psychrophilic phototrophs to date. In contrast, the *Chlorella* sp. resides as part of a microbial mat community in a transient pond located near Bratina Island in McMurdo Sound. The natural environment of the *Chlorella* sp. is highly divergent from the Dry Valley lake environment of *C. raudensis*, and is characterized by periods of high light and UV exposure, desiccation events, as well as seasonally variable environmental stress conditions. Currently, I am conducting photophysical and basic physiological studies in these psychrophilic algae to gain understanding about the combinatory effects of low temperature and variable environmental stress on photosynthetic adaptation. Upcoming projects will involve enzymological characterization of the RubisCO holoenzyme and sequencing of the chloroplast genome from both psychrophiles.

Another goal of my research is to characterize the phototrophic community diversity from extreme environments. Currently, I am working on vertical distribution of phototrophs in the four main lakes in McMurdo Long Term Ecological Research (McM-LTER) site. The McM-LTER is one of world-wide LTER locations

and represents an 'end member' in the group, being the driest and coldest LTER site in the world. Phototroph biodiversity is assessed using independent analyses such as phylogenetics as well as distribution of FAMES and pigment biomarkers. The results of this study will be ultimately used to verify the validity of a newly acquired method of the McM-LTER to assess phytoplankton biodiversity in situ. This method relies on differences Chlorophyll a fluorescence excitation of spectral algal groups, using the BBE FluoroProbe (BBE Molandecke, Germany).

In a new project, we will be returning to the Dry Valley Lakes in the winter of 2008. This work will be part of a collaborative effort to study the microbial populations during the transition from the Antarctic austral summer to winter. My laboratory will be conducting photosynthetic gene expression studies on the native phytoplankton populations as well as studying the functional and structural changes in the photochemical apparatus in monocultures of *C. raudensis*, which will be transplanted from the laboratory to its native environment. This project will be the first study the Dry Valley Lake populations during the summer to winter transition as well as attempt to link our understanding of a psychrophilic phototroph under controlled laboratory conditions with its response to its natural environment.

Selected Publications

Morgan-Kiss R.M., Priscu JC, Pockock T, Gudnait-Savich L., Hüner NPA (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. *Microbiol. Molec. Biol. Rev.* 70:222-252

Morgan-Kiss R.M., Ivanov A. G., Pockock T., Gudnait-Savitch L., Hüner N. P. (2005) The Antarctic psychrophile, *Chlamydomonas raudensis* Ettl (UWO241) (Chlorophyceae, chlorophyta) exhibits a limited capacity to photoacclimate to red light. *J Phycol.* 41 (4): 791-800

Morgan-Kiss R.M., Cronan Jr. JE (2004) The *Escherichia coli fadK (ydiD)* gene encodes an aerobically-regulated short-chain acyl-CoA synthetase. *J. Biol. Chem.* 279:37324-37333.

Morgan-Kiss R.M., Wadler C, Cronan Jr., JE (2002) Long-term and homogeneous regulation of the *Escherichia coli araBAD* promoter by use of a lactose transporter of relaxed specificity. *Proc. Natl. Acad. Sci. USA* 99:7373-7377.

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JOHN R. STEVENSON

Associate Professor of Microbiology

Research Interests

I am interested in the effects of protein malnutrition on development and function of the immune system. Studies in my laboratory have shown that protein malnourished mice develop an immunodeficiency syndrome characterized by thymic atrophy that leads to depressed immune responsiveness. Development of this immunodeficiency syndrome is triggered by increased plasma corticosterone, which induces thymocyte apoptosis that results in thymic atrophy. As malnutrition continues, the number of T lymphocytes decreases in the blood and the spleen as well as in the thymus. This immunodeficiency syndrome is important to study because T cells play a pivotal role in generation and maintenance of the immune responses necessary for prevention of, and recovery from, infection.

Current research in my laboratory is aimed at determining how this immunodeficiency syndrome develops and how it affects the functions of the immune system in host defense against infection.

We have shown that high levels of corticosterone induce apoptosis of immature thymic T cells to cause the thymic atrophy seen in protein malnourished mice. However, the thymic atrophy is not as rapid nor as severe as would be predicted based on the corticosterone levels in these mice. This is because apoptosis is somehow downregulated by heat shock proteins produced by these thymocytes in response to the stress of their protein deficiency. We are now investigating the mechanisms by which the heat shock proteins inhibit thymocyte apoptosis as well as the mechanisms by which corticosterone induces apoptosis in this system. These studies are facilitated by the use of silencing RNA and plasmid constructs encoding heat shock protein genes, flow cytometric analysis of apoptotic events, and intracellular flow cytometric assays of glucocorticoid receptors and heat shock proteins.

Our protein malnourished mice exhibit decreased resistance

to infection with bacteria such as *Listeria monocytogenes* and *Salmonella enteritidis*, and by the fungus, *Candida albicans*. We have shown that corticosterone causes decreased killing of *Candida* by neutrophils in these mice in addition to slowing their ability to form abscesses, and we think this is due to diminution of T cell numbers below the level needed to produce the cytokines necessary to enhance host defenses. We have shown that the decreased resistance to *Salmonella* is due to a combination of low T cell numbers, which leads to diminished ability to synthesize enough interferon-gamma for efficient activation of macrophages, and direct inhibitory effects of corticosterone on macrophage activation. To gain more insight into the mechanisms by which these effects occur, we are investigating the effects of corticosterone on macrophage synthesis/secretion of interleukin-12 (IL-12) and T cell synthesis/secretion of IL-4, IL-10 and interferon-gamma. We are correlating the levels of cytokines generated in our spleen cell based *in vitro* system with the degree of macrophage activation observed. These studies are facilitated by the use of cytokine array analysis kits and flow cytometric quantification macrophage activation.

Selected Publications

Davis, N.J., and **J.R. Stevenson**. 2003. Role of Neutrophils in Decreased Resistance of Protein-Malnourished Mice to *Candida albicans*. *Nutr. Res.* 23:945-958.

Barone, K.S., and **J.R. Stevenson**. 1994. Characterization of thymic atrophy and regeneration in protein-malnourished weanling mice. *J. Nutr. Immunol.* 3:13-26.

Barone, K.S., P.C.M. O'Brien, and **J.R. Stevenson**. 1993. Characterization and mechanisms of thymic atrophy in protein-malnourished mice: role of corticosterone. *Cell. Immunol.* **148**:226-233.

Mellencamp, M.W., P.C.M. O'Brien, and **J.R. Stevenson**. 1991. Pseudorabies virus-induced suppression of MHC class I antigen expression. *J. Virol.* **65**:3365-3368.

MARY WOODWORTH

Professor of Microbiology

Research Interests

By utilizing molecular techniques and a small tumor virus (SV40) as our model system, we are investigating how replication is controlled and its implications for uncontrolled cell growth in cancer. Naturally arising variants of SV40 which have evolved because of their selective advantage over wild type and other defectives are being utilized to elucidate the mechanisms of initiation of replication in eukaryotic cells. By a comparative analysis of the biological activities of cloned variant sequences that during evolution have replaced regulatory wild type sequences, we hope to identify cis-acting sequence elements and trans-acting factors which positively or negatively regulate replication.

Current Projects

It is particularly interesting that the monkey DNA sequences in naturally arising variants of SV40 share no sequence homology with SV40 regulatory regions yet both can lead to either increased or decreased replication efficiency depending on their location relative to the SV40 origin of replication (*ori*). The molecular mechanisms that determine the site at which eukaryotic DNA replication is initiated are not known. We plan to map replication initiation sites in our various plasmid constructs containing rearranged regulatory elements (from monkey and SV40 DNA) in order to test the hypothesis that replication efficiency is correlated with the site of replication initiation.

Furthermore, the analysis of cis-acting motifs that enhance replication has implicated certain cellular transcription factors. Protein-binding experiments performed with crude extracts and also with purified proteins will be used to identify factors and define the nucleotides within the monkey sequence with which they interact. The ability of factors to facilitate T antigen-mediated DNA unwinding will be tested directly by probing with a chemical that can detect alterations in DNA conformation, such as potassium permanganate. We will also determine whether there is a relationship between nucleosome ordering and the differences in chromosomal DNA replication efficiency that are based on the location of regulatory sequences relative to *ori*.

In parallel with the binding experiments, further cis-acting studies will be done. Point mutations in binding sites will be made by site-directed mutagenesis so that the individual motifs can be assayed without altering the remainder of the regulatory sequence. Another line of experiments will assay the replication activity of reiterated binding sites to determine if the stimulation of replication conforms to the enhancer model of transcriptional activation.

Selected Publications

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Turner, W.J. and **M.E. Woodworth**. 2001. DNA replication efficiency depends on transcription factor-binding sites. *J. Virol.* **75**:5638-5645.

Wilderman, P.J., B. Hu, and **M.E. Woodworth**. 1999. Conformational changes in simian virus 40 rearranged regulatory regions: effects of the 21-bp promoters and their location. *J. Virol.*, **73**:10254-10263.

Adiletta, D.C., R.W. Elliott, and **M.E. Woodworth**. 1993. Characterization of murine middle repetitive DNA. *DNA and Cell Biol.* **12**:319-327.