Tuesday September 4th – Gray / Palmer / Pope
Bacteriology / Mycology 3
Moderator – Ben LaFrentz (USDA – Agricultural Research Service)

1:15 PM	Bacteriology 3	Gulla - Detection and Epizootiology of *Yersinia ruckeri*
1:30 PM		Kumar - Proteomic Changes of Rainbow Trout Intestinal Mucosa in Response to *Yersinia ruckeri*
1:45 PM		Menanteau-Ledouble - Host Genes Involved in Intracellular Invasion by the Enterobacterium *Yersinia ruckeri* in Fish Cell Cultures
2:00 PM		Welch - Flagellar Regulation Is Required for Virulence in *Yersinia ruckeri*
2:15 PM		Katharios - *Aeromonas veronii* bv *sobria*: An Emerging Threat for European Seabass Aquaculture. Virulence and Vaccination Trials
2:30 PM		**Richardson - Evaluating Atypical *Aeromonas hydrophila* (aAh) in Catfish Aquaculture of the Mississippi Delta Region**
2:45 PM		Xiao - A new Isolate of *Aeromonas salmonicida* Caused Furunculosis in Atlantic Salmon (*Salmo salar*) From Recycling Aquaculture System in China

Detection and Epizootiology of *Yersinia ruckeri*

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Yersinia ruckeri is a significant pathogen of farmed salmonid fish worldwide. Following its first identification in Norwegian farmed salmon in 1985, yersiniosis rapidly became a serious problem with nearly sixty farms affected in 1987. By the mid-90's, however, the number of annual outbreaks were drastically reduced, largely through improved production conditions and increased focus on biosecurity. The situation remained stable until the mid-2000's when the number of outbreaks yet again began to increase, both in freshwater and marine sites.

While the bacterium can easily be cultured and serotyped using standard techniques, or detected by PCR, diagnostic investigations have not always correlated well with the clinical yersiniosis situation, possibly indicating strain differences in virulence. To improve our understanding of the epizootiology of yersiniosis we have focused our research on molecular typing and improved PCR detection of virulent *Y. ruckeri* infections. We have developed both a high-resolution Multi-Locus Variable number of tandem repeat Analysis (MLVA) scheme and a series of specific qPCR assays for *Y. ruckeri* detection at the species, serotype and clonal complex levels.

Our research has revealed that nearly all yersiniosis outbreaks in Norwegian aquaculture over the last twenty years or so have been caused a single clonal complex of serotype O1, apparently exclusive to Norway. Similarly, distinct clones appear to dominate in other salmon-producing countries, while another serotype O1 clonal complex dominates the disease situation in international rainbow trout farming. While most historic and present yersiniosis outbreaks worldwide have been associated with serotype O1 strains, we find an increasing body of evidence for the widespread existence of putatively low-virulent or avirulent environmental strains belonging to serotype O1.

Conference Session Designation: (Bacteriology / Mycology)
Presentation Format: (Oral)

Proteomic Changes of Rainbow Trout Intestinal Mucosa in Response to *Yersinia Ruckeri*

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Yersinia ruckeri is the causative agent of enteric redmouth disease in salmonids. The signs of the disease include exophthalmia, subcutaneous hemorrhages, splenomegaly and inflammation of the lower intestine. The intestinal epithelium is a primary site of enteric pathogens infection. The intestine is a multifunctional organ that plays a crucial role in nutrient uptake, host–pathogen interactions and immune system. Little is known about intestinal proteomic changes in rainbow trout in response to *Y. ruckeri*. In this study, we examined proteome changes in the intestine of rainbow trout after exposure to *Y. ruckeri*. Fish were challenged by immersion of *Y. ruckeri* strains and sampled at different time points. Each lower intestine was opened and washed three times with phosphate-buffered saline. Intestinal mucosa was scraped and immediately frozen in liquid nitrogen. Intestinal mucosa samples were resuspended in denaturing lysis buffer and disrupted by sonication. The lysates were centrifuged and supernatants were collected. Protein digestion was performed using a standard in-solution protocol. Resulting peptides were analyzed by nano LC-MS/MS directly coupled to a high resolution quadrupole time of flight mass spectrometer (TripleTOF 5600). Quantification of proteins was performed using SWATH MS2 data independent technology. Statistical analysis was performed in R programming language to identify differential expression of intestinal mucosa proteins. Sophisticated statistical evaluation revealed 62 up-regulated and 75 down-regulated proteins in intestinal mucosa of rainbow trout during *Y. ruckeri* infection. These proteins mostly are related to exopeptidase and endopeptidase activities, defense response, ion binding and metabolic process. The findings of this study provide new insight to understanding defence mechanisms and host-pathogen interactions of intestine during *Y. ruckeri* infection. These advanced proteomic data expand our knowledge on effects of *Y. ruckeri* on intestine of rainbow trout.

Conference Session Designation: (Bacteriology)
Presentation Format: (Oral)

Host Genes Involved in Intracellular Invasion by the Enterobacterium *Yersinia Ruckeri* in Fish Cell Cultures

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Yersinia ruckeri is an important fish pathogen. Like other members of the genus *Yersinia*, it is a facultative intracellular bacterium. While little is known about the mechanisms of intracellular invasion in *Y. ruckeri*, several mechanisms of invasion have been described in other bacterial pathogens, including within the genus *Yersinia*. Interestingly, the bacterium triggers its own uptake by the host cells and therefore relies on the apparatus of the host cells, for example, its cytoskeleton.

Consequently, the role of several host genes in the invasion process of *Y. ruckeri* was investigated. 17 genes that are known to play a role in the invasion of other facultative intracellular bacterial pathogens were silenced in Chinook Salmon Embryo using small inhibitory RNA. The cells were then exposed to *Y. ruckeri* and their susceptibility to infection was assessed using a gentamycin assay.

Inactivation of all 17 genes resulted in a decreased number of bacteria recovered at the end of the assay. However, in only 13 of these 17 genes were the differences statistically significant (Sumo 2, CDC 42, arhgap18 and, β-cadherin Actin were not statistically significant). The fact that multiple genes appear required for the invasion of *Y. ruckeri* is consistent with our previous findings that this pathogen makes use of multiple mechanisms of entry into the host.
The present results will contribute to our understanding of the virulence mechanisms in *Y. ruckeri* and in particular of the bacterium's interactions with the host cells.

Conference Session Designation: (Bacteriology / Mycology)
Presentation Format: **(** Oral)

Flagellar Regulation is Required for Virulence in *Yersinia ruckeri*

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The gram-negative Enterobacterium *Yersinia ruckeri* is the etiologic agent of enteric redmouth disease (ERM), a septicemia affecting primarily farmed rainbow trout (*Oncorhynchus mykiss*, Walbaum). Expression of the flagellin locus (*fliC)* is repressed during the course of infection and subsequently up-regulated upon host mortality in a motile strain of *Y. ruckeri*. We have recently used a selective method to identify a spontaneous *Y. ruckeri* mutant strain (TW32) that displays elevated and constitutive expression of the flagellar motility phenotype. Strain TW32 is non-virulent and exposure of rainbow trout to this strain induces a specific anti- *Y. ruckeri* IgM antibody response and non-specific anti-*Flavobacterium pschrophillum* immunity of unknown duration. Virulence in TW32 is restored when the flagellar secretion system is inactivated through mutation of the *filR* gene in the TW32 background. This demonstrates that the attenuating mutation in TW32 exerts its effect through the flagellar secretion system and is thus dependent on either a component of the system (flagellin) or a secreted factor. Genome sequencing of the TW32 strain and marker-exchange experiments revealed that a single mutation in the promoter region of the flagellar master regulator *FlhDC* is responsible for this phenotype. These results suggest that the inappropriate expression of flagellar secretion during infection triggers host recognition and thus immune stimulation resulting in attenuation of virulence. The repression of flagellin expression during infection likely occurs in order to evade host recognition and is critical for *Y. ruckeri* virulence. We also hypothesize that these unique properties of TW32 could make this strain an ideal live-attenuated vaccine.

Conference Session Designation: (Bacteriology / Mycology)
Presentation Format: **(** Oral)

Aeromonas Veronii bv _Sobria_: An Emerging Threat for European Seabass Aquaculture. Virulence and Vaccination Trials

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European seabass, *Dicentrarchus labrax* is one of the most important species for the Mediterranean Aquaculture. The past years, morbidity and mortality of cage-cultured seabass due to infections by *Aeromonas veronii* bv *sobria* have been reported from Greece and Turkey which are the main producers of the species. More than 50 strains of the pathogen were isolated from various locations in Greece and Turkey and were partially characterized. The genomes of nine strains were fully sequenced. These strains were representative of the geographic origin of isolation, but also of the phenotypes of the bacteria since isolates have differences in motility and pigment production. Virulence of the sequenced strains was examined in adult zebrafish where LD50 values ranged between 4.3×10^5 – 1.3×10^6 cfu/fish at 24h following intraperitoneal (i.p.) administration. Differences in the phenotypes and virulence of the strains were studied through comparative genomic analysis. Two of the sequenced strains were tested in adult seabass, in which virulence was significantly higher. Following a 2.5h immersion challenge in 10^5 cfu/mL, seabass suffered mortality of 100% within 7 and 10 days post challenge for the two strains, respectively. The two strains were used as the basis of a bivalent autogenous vaccine using Montanide ISA 763 A VG as an adjuvant. Following i.p. vaccination and subsequent immersion challenge with *Aeromonas veronii* bv *sobria* 30 days post vaccination, the autogenous vaccine resulted in high protection with RPS_{60} equal to 100%. Vaccination could be the method of choice for the management of disease, however the variance in the phenotypic and genomic traits of the bacterial strains indicates that a cautious choice of the appropriate antigen is required to achieve a global protection.

Conference Session Designation: **(** Bacteriology / Mycology or Genomic Applications)
Presentation Format: (Oral)

Evaluating Atypical *Aeromonas hydrophila* (aAh) in Catfish Aquaculture of the Mississippi Delta Region

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Atypical *A. hydrophila* (aAh) has been plaguing channel catfish *Ictalurus punctatus* aquaculture farms in the southeastern US since the late 2000s. Multiple serotypes of aAh effect various parts of Alabama and Mississippi, and clinical symptoms vary with serotype. Our study aimed to investigate the status of aAh in catfish aquaculture ponds of Mississippi. Number of aAh outbreaks varies between years, and anecdotal evidence suggests some temperature-dependence. Water samples and culture swabs were collected from disease and non-diseased ponds. Once notified of a potential aAh outbreak from a farm manager, samples were collected from the infected pond, as well as one adjacent pond and one non-adjacent pond. Samples were subjected to quantitative polymerase-chain reaction (qPCR) to determine aAh pathogen load within each sample. Pond outbreaks appeared random, and in most cases, only one pond showed signs of an active outbreak, at any given time. Using occupancy models, our results showed as much as 60 % or more of the population of a pond may be infected with aAh with no visual signs of disease outbreak. The results of this study suggest aAh outbreaks in catfish aquaculture ponds are not isolated incidences, but that multiple ponds may be infected, making outbreak prediction more difficult. Ongoing studies are focusing on outbreak predictors, such as environmental drivers, and possible vaccines. At this point, only one antibiotic is available for treating aAh outbreaks. This lack of treatment options increases the risk of antibiotic resistance in the pathogen and could exasperate the issue even further.

Conference Session Designation: **(** Emergent Disease)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

A New Isolate of *Aeromonas salmonicida* Caused Furunculosis in Atlantic salmon (*Salmo salar*) from Recycling Aquaculture System in China

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Atlantic salmon (*Salmo salar*) is the most successful cultured fish species in world aquaculture. Considering of its rich nutrition and good taste as an excellent ingredient, a Chinese company imported fertilized eggs of Atlantic salmon from Norway and started hatching and culturing the fish in 2010. That was the first attempt to culture Atlantic salmon in land based recirculating aquaculture systems both in freshwater and seawater period. Years later, disease became a predominant restriction for the industry. We isolated many bacterial isolates from diseased Atlantic salmon samples and they were all identified as *Aeromonas salmonicida* by molecular methods. Physiological and biochemical characteristics results showed that the isolates were *Aeromonas salmonicida* subsp. *masoucida*. In vitro cultured bacteria induced furuculosis like symptoms in Atlantic salmon. And the bacteria could be re-isolated from these infected fish. The clinical isolate performed strong virulence to Atlantic salmon when been intramuscularly injected into experimental fish. The LD50 was $9.67\times10^{2.18}$ CFU/fish. These findings showed that an *A. salmonicida* subsp. *masoucida* was the causative agent of the furuculosis like disease as each of Koch's postulates were fulfilled. An inactivated vaccine was prepared and it provided protection with relative protection percentages of 80% against C4 and 40% against NCIMB1102 respectively. This was the first report of *A. salmonicida* subsp. *masoucida* causing Atlantic salmon with furuculosis in recirculating aquaculture system in China. And the vaccine developed in this study protected Atlantic salmon against furuculosis.

Conference Session Designation: (Immunology Vaccines)
Presentation Format: (Oral)