Monday September 3rd – Archibald / Campbell
Flavobacterium 1 & 2
Moderator - Tom Loch (Michigan State University)

9:30 AM	Flavobacterium 1	Knupp - Novel MLST *F. psychrophilum* Genotypes Infecting North American Salmonids
9:45 AM		Beka - Detection of aquaculture pathogens *Flavobacterium columnare* and *F. psychrophilum* using 16S rRNA amplicon sequencing and high-resolution sequence variant typing
10:00 AM		LaFrentz - Identification Of Four Distinct Phylogenetic Groups In *Flavobacterium columnare* With Fish Host Associations
10:15 AM		**Cai - Unveiling The Genetic Diversity Behind The Species Complex *Flavobacterium columnare***
10:30 AM		**Refreshments**
10:45 AM	Flavobacterium 2	**Nakajima - Resistance against *Flavobacterium psychrophilum* in Ayu *Plecoglossus altivelis* Hatchery-Reared at Different Water Temperatures**
11:00 AM		**Sebastião - Characterization of *Chryseobacterium* spp. isolated from clinically affected fish in California**
11:15 AM		**Hu - Infectious disease caused by Elizabethkingia in farmed frogs, China**
11:30 AM		**Klakegg - Isolation, identification and characterization of a *Tenacibaculum dicentrarchi* like bacteria causing acute disease and mortality in Atlantic salmon in a Norwegian post smolt site.**
11:45 AM		Marsh - Natural antibiotic sensitivity and biofilm formation in Flavobacterium

Novel MLST *F. psychrophilum* Genotypes Infecting North American Salmonids

Christopher Knupp[1], Gregory Wiens[2], Mohamed Faisal[1,3], Douglas Call[4], Kenneth Cain[5], Pierre Nicolas[6], Danielle Van Vliet[7], Coja Yamashita[8], Jayde Ferguson[9], David Meuninck[10], Hui-Min Hsu[10], Bridget Baker[11], Ling Shen[12] and Thomas Loch[1,3]*

[1] Department of Fisheries & Wildlife, MSU, East Lansing MI USA knuppch1@msu.edu
[2] United States Department of Agriculture – ARS, Kearneysville, WV, USA
[3] Department of Pathobiology & Diagnostic Investigation, Michigan State University, East Lansing, MI USA
[4] Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA USA
[5] Department of Fish and Wildlife Sciences, University of Idaho, Moscow, ID USA
[6] MaIAGE, INRA, Université Paris-Saclay, Jouy-en-Josas, France
[7] Utah Division of Wildlife Resources, Logan, UT USA
[8] Fish Health/Pathology Unit, Pennsylvania Fish & Boat Commission, State College, PA ,USA
[9] Alaska Department of Fish and Game, Anchorage, AK USA
[10] Indiana Division of Fish and Wildlife, Mishawaka, IN USA
[11] Division of Laboratory Animal Resources, Wayne State University, Detroit, MI USA
[12] Fish and Wildlife Division, Minnesota Department of Natural Resources, St. Paul, MN USA

Flavobacterium psychrophilum is the etiological agent of bacterial coldwater disease (BCWD) and is responsible for significant economic losses in salmonid aquaculture worldwide, particularly farm-raised rainbow trout (*Oncorhynchus mykiss*). Over the last decade, multiple researchers have investigated the genetic heterogeneity of this bacterium in Europe, Asia, South America, Oceania, and most recently, North America (NA), using multilocus sequence typing (MLST), which linked some genetic variation to geographic range, host specificity, and association with BCWD outbreaks. However, much remains unknown regarding the population structure of *F. psychrophilum* in the USA, a matter of concern for disease prevention and control. Therefore, MLST was used to genotype 314 North American *F. psychrophilum* isolates, which were recovered from 10 fish host species in 20 US states and 1 Canadian province over nearly four decades. Results revealed these isolates belonged to 66 sequence types (STs), 47 of which were novel. Furthermore, 7 novel NA CCs were discovered, which brings the total number of NA CCs to 12. Many of the identified CCs have only been detected in NA to date, whereas others have been recovered from NA and abroad. These CCs were diverse and varied in terms of host specificity, distribution, and association with BCWD outbreaks. The largest *F. psychrophilum* CC identified in this study was CC-ST10, whereby 10 novel genotypes were detected and primarily recovered from BCWD epizootics in *O. mykiss*. ST275 of CC-ST10 was recovered from wild/feral adult steelhead trout (*O. mykiss*) and the recovery of CC-ST10 from feral/wild fish in NA has not been reported previously. Furthermore, the progeny from these fish were found to harbor the same ST, thereby supporting that some *F. psychrophilum* strains are circumventing current egg disinfection techniques. Ongoing experiments are exploring how such diversity relates to *in vivo* virulence and *in vitro* antibiotic susceptibility. Study findings to date will be invaluable in devising improved and targeted prevention and control strategies to reduce BCWD-associated losses.

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

Detection of Aquaculture Pathogens *Flavobacterium columnare* and *F. psychrophilum* Using 16S Rrna Amplicon Sequencing and High-Resolution Sequence Variant Typing

Lidia Beka*[1], Emily Ann McClure[1], Todd Testerman[1], Erin Breaker[2], Ahmad Hassan[1], Susan Janton[1], Randy MacMillan[3], Greg Wiens[4], Tim Welch[4], Joerg Graf[1]

[1] University of Connecticut, Storrs, CT, USA Lidia.Beka@uconn.edu
[2] Centers for Disease Control and Prevention, Atlanta, GA, USA
[3] Clear Springs Foods, Inc., Idaho, USA
[4] USDA-ARS, Leetown, WV

Flavobacterium columnare and *F. psychrophilum* cause high levels of mortality in the aquaculture industry and methods of detecting these pathogens in a facility are invaluable for the prevention of future outbreaks. Here, we use 16S rRNA amplicon deep sequencing and oligotyping to determine the presence of *F. columnare* and *F. psychrophilum*-specific sequences in a trout farm in the US. Water flowing into and out of the trout farm raceways was collected and filtered. Gill and intestinal samples were also collected from morbid and healthy fish in these raceways. DNA was extracted from water filters and fish tissue samples. The V4 region of the 16S rRNA gene was sequenced on an Illumina MiSeq. Single-nucleotide variant reads, SNVs, were identified and taxonomically assigned using the DADA2 analysis package with the Silva reference database. The identification was confirmed by aligning the SNVs to reference type strain sequences and those that matched 99.99-100% to pathogen reference sequences were quantified. Presence and absence results of pathogen-specific sequences in various sample types from the farm were further verified using a droplet digital PCR (ddPCR) analysis, which targeted a different genetic marker. This approach allowed us to discriminate between and determine the relative abundance of *F. columnare* and *F. psychrophilum* sequences in samples collected at this farm over two years. *F. psychrophilum* and *F. columnare* were detected in water entering the raceways, in gill samples from sick fish (up to 1,764 sequences per 10,000 reads), and in swabs from baffles placed within the raceways. This method not only allows us to determine the microbial community composition of various samples but also shows great potential for identifying *Flavobacterium* pathogens at aquaculture facilities. Amplicon sequencing allows us track the source of these infectious agents and importantly, to survey raceways before an outbreak occurs which can be useful for deciding on an intervention.

Conference Session Designation: (Flavobacteria / Diagnostics)
Presentation Format: (Oral or Poster)

Identification of Four Distinct Phylogenetic Groups in *Flavobacterium columnare* with Fish Host Associations

Benjamin R. LaFrentz[1*], Julio C. García[1], Geoffrey C. Waldbieser[2], Jason P. Evenhuis[3], Thomas P. Loch[4], Mark R. Liles[5], Fong S. Wong[6], Siow F. Chang[6]

[1] USDA-ARS, Aquatic Animal Health Research Unit, 990 Wire Road, Auburn, AL 36832 USA benjamin.lafrentz@ars.usda.gov
[2] USDA-ARS, Warmwater Aquaculture Research Unit, Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS USA
[3] USDA-ARS, National Center for Cool and Cold Water Aquaculture, Kearneysville, WV USA
[4] Michigan State University, Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, East Lansing, MI USA
[5] Auburn University, Department of Biological Sciences, Auburn, AL USA
[6] MSD Animal Health Innovation Pte Ltd, Singapore

Columnaris disease, caused by the Gram-negative bacterium *Flavobacterium columnare*, is one of the most prevalent fish diseases worldwide. An exceptionally high level of genetic diversity among isolates of *F. columnare* has long been recognized, whereby six established genomovars have been described to date. However, little has been done to quantify or characterize this diversity further in a systematic fashion. The objective of this research was to perform phylogenetic analyses of 16S rRNA and housekeeping gene sequences to decipher the genetic diversity of *F. columnare*. Fifty isolates and/or genomes of *F. columnare*, originating from diverse years, geographic locations, fish hosts, and representative of the six genomovars were analyzed in this study. A multilocus phylogenetic analysis (MLPA) of the 16S rRNA and six housekeeping genes supported four distinct *F. columnare* genetic groups. There were associations between genomovar and genetic group, but these relationships were imperfect indicating that genomovar assignment does not accurately reflect *F. columnare* genetic diversity. To expand the dataset, an additional ninety 16S rRNA gene sequences were retrieved from GenBank and a phylogenetic analysis of this larger dataset also supported the establishment of four genetic groups. Examination of isolate historical data indicated biological relevance to the identified genetic diversity, with some genetic groups isolated preferentially from specific fish species or families. It is proposed that *F. columnare* isolates be assigned to the four genetic groups defined in this study rather than genomovar in order to facilitate a standard nomenclature across the scientific community. An increased understanding of which genetic groups are most prevalent in different regions and/or aquaculture industries may allow for the development of improved targeted control and treatment measures for columnaris disease.

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

Unveiling the Genetic Diversity Behind the Species Complex *Flavobacterium columnare*

Wenlong Cai* and Cova R. Arias

School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, Auburn, AL 36849 USA wzc0017@tigermail.auburn.edu ariascr@auburn.edu

Flavobacterium columnare is the causative agent of columnaris disease, which causes significant losses in cultured freshwater finfish species across the world. The instraspecies genetic diversity found in *F. columnare* was first revealed by DNA-DNA hybridization that divided the species into 3 genetic groups or genomovars. Later studies further subtyped the species into 5 genetic groups based on 16S rDNA polymorphisms. Virulence studies in channel catfish showed that genovar II strains were more virulent than genomovar I strains, suggesting the presence of more than one patovar within the species. On the contrary, all *F. columnare* strains are biochemically similar. The objective of this study was to elucidate if *F. columnare* was a species complex that harbors more than one cryptic species or if the observed genetic diversity was within the definition of bacterial species. Based on polyphasic data previously obtained by our group, we selected three strains representing 3 different lineages within the species (ATCC 23463 (type strain), ARS-1, and BGFS-27) for whole genome sequencing using PacBio RS long-read sequencing platform. *De novo* genome assembly of filtered reads was performed using PacBio PBcR HGAP 2.3 pipeline with default settings, which yielded 5 (ATCC 23463), 7 (ARS-1), and 16 (BGFS-27) contigs with 214x, 182x, and 184x coverage, respectively. Average nucleotide identity (ANI) were 85.55%, 85.69, and 91.3% for groups ARS1 & ATCC 23463, ARS1 & B27, and B27 & ATCC 23463, respectively. All ANI values were lower than the recommended cut-off point of 95% for species delineation. ANI results validated previous MLST and MALDI-TOFF phylogenetic analyses. Comparative genomic analysis (CGA) identified 1,876 genes in the core genome (shared by all 8 strains), which accounted for 34.2% of the total pangenome (gene repertoire= 5,491 genes). Strains within lineage 1 (represented by ATCC 23463) contained 61 unique genes while lineage 3 (represented by ARS-1) harbored up to 459 unique genes. Lineage 2 (represented by BGFS-27 and highly virulent for catfish) contained 52 unique genes including several genes encoding for putative virulence factors (O-antigen polymerase, glycosyltransferase, streptococcal hemagglutinin protein, type II toxin-antitoxin system toxin, and subtilisin-like serine protease). Based on our data, three species of *Flavobacterium* can cause columnaris disease in fish: *F. columnare* and two nomen nudum species that warrant full taxonomic description. Our results have direct implications in control and prevention of columnaris disease in farms.

Conference Session Designation: (Bacteriology / Flavobacteria)
Presentation Format: (Oral)
Student Presentation: (Yes)

Resistance against *Flavobacterium psychrophilum* in Ayu *Plecoglossus altivelis* Hatchery-Reared at Different Water Temperatures

Hayato Nakajima[1]*, Kyuma Suzuki[2], Osamu Kurata[3], Kosei Taguchi3, Motohiko Sano[1], Goshi Kato[1]

[1] Department of Marine Biosciences, Tokyo University of Marine Science and Technology,
Tokyo 108-8477, Japan gkato00@kaiyodai.ac.jp
[2] Gunma Prefectural Fisheries Experiment Station, Gunma 371-0036, Japan
[3] Laboratory of Fish Disea ses, Nippon Veterinary and Life Science University, Tokyo 1800023, Japan

Ayu *Plecoglossus altivelis* is one of the most important fish species in Japanese inland water fishery; however, several pathogenic bacteria cause mass mortalities of the cultured and wild fish. Although rearing at high water temperatures (17°C–20°C) promotes growth of ayu seedlings, thymus development is remarkably inhibited under the high water temperature condition. In this study, we aimed to investigate disease resistance of ayu reared at different water temperatures against *Flavobacterium psychrophilum*, the pathogen of bacterial cold-water disease. Ayu (mean body weight = 0.85 g) were reared at 12°C or 18°C for 2 months, followed by acclimatization at 15°C for 1 month. Cubic volume of the thymus was measured by computed tomography scanning. Fish were intraperitoneally injected with *F. psychorphilum* (1.6 × 107 CFU/fish and 6.6 × 106 CFU/fish), and the cumulative survival rate was statistically analyzed with Log-rank test. Furthermore, the trunk kidney was sampled from the infected fish at 0, 1 and 2 days after the infection, and gene expression analysis of IL-1β and in the trunk kidney was performed. The thymus volume of 18°C group was significantly lower than that of 12°C group ($p < 0.01$), when the rearing at 12°C or 18°C was over. Although all experimental fish died in both 12°C and 18°C groups, the survival times of 12°C group were significantly longer than 18°C group in high-dose and low-dose challenge ($p < 0.05$). Gene expression level of IL-1β was significantly up-regulated at 1 day post-infection compared with uninfected fish in 12°C group, whereas significant difference was not observed in the gene expression level in 18°C group. These results suggest that rearing at the high-water temperature negatively influences on resistance of ayu to *F. psychrophilum.* Further, fish reared at the high-water temperature could not induce normal inflammatory responses to the infection.

Conference Session Designation: (Immunology / Flavobacteria)
Presentation Format: (Oral)
Student Presentation: (Yes)

Characterization of *Chryseobacterium* spp. Isolated From Clinically Affected Fish in California

Fernanda de Alexandre Sebastiao[1], Matt J. Griffin[2], Dave Marancik[3], Thomas P. Loch[4], Esteban Soto[1]

[1] Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA dealexandresebastiao@ucdavis.edu sotomartinez@ucdavis.edu.

[2] College of Veterinary Medicine, Mississippi State University, 127 Experiment Station Road P.O. Box 197, Stoneville, MS 38776, USA. griffin@cvm.msstate.edu.

[3] Department of Pathobiology, School of Veterinary Medicine, St. George's University, True Blue, St. George's Grenada, West Indies DMaranci@sgu.edu

[4] Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA lochthom@msu.edu

Some members of the family *Flavobacteriaceae* are recognized as emergent fish pathogens, including *Chryseobacterium* spp. In this study, seven bacterial strains recovered from 2015-2018 from spleen of diseased rainbow trout, *Oncorhynchus mykiss* (*n*=1), green sturgeon, *Acipenser medirostris* (*n*=1), white sturgeon, *Acipenser transmontanus* (n=2), blue cichlid (n=1) and fall chinook salmon, *Oncorhynchus tshawytscha* (n=2) were characterized by phenotypic and molecular taxonomic methods. After 24-48 hrs incubation at 20°C, colonies on tryptone-yeast extract-salts agar media were yellow, mucoid, circular in shape with entire margins. The isolates were Gram negative, rod-shaped, catalase and oxidase positive. Amplification and partial sequence analysis of 900 bp of the 16S rRNA gene allocated the microorganisms to the genus *Chryseobacterium*, with isolates presenting 98.1%, 99.6%, 97.5%, 97.2% 98.9% and 99.7% homology to *C. viscerum*, *C. aquaticum*, *C. sediminis*, *C. culicis*, *C. ureilyticum*, and *C. indologenes*, respectively. In order to investigate the pathogenicity of the recovered isolates, five isolates (e.g, *C. viscerum*-like, *C. aquaticum*-like, *C. sediminis*-like, *C. culicis*-like, and *C. indologenes*-like) were selected to challenge rainbow trout, brown trout *Salmo trutta* and white sturgeon under laboratory conditions. Fish were acclimated and challenged in flow-through freshwater at 18°C. Approximately $5x10^7$ CFU/fish of each *Chryseobacterium* strain were intramuscularly injected in the epaxial musculature of anesthetized animals (n=10 per treatment). No mortality occurred in fish challenged with *C. aquaticum*-like, *C. sediminis*-like, and *C. indologenes*-like isolates. White sturgeon exposed to the *C. viscerum*-like strain, and brown trout exposed to *C. culicis*-like strain experienced 10% mortality (1/10). However, the bacterium was not reisolated from the posterior kidney of these fish. Thirty days post-challenge, survivors were euthanized and multiple tissues were collected and fixed for histopathological analysis. Although results suggest that the recovered *Chryseobacterium* sp. may be opportunistic microorganisms, further research is warranted to better understand the role of these bacteria in fish diseases.

Conference session designation: **(** Flavobacteriaceae)
Presentation format: (Oral)
Student presentation: (Yes)

Infectious Disease Caused by *Elizabethkingia* in Farmed Frogs, China

Ruixue Hu, Qi Zhang and Zemao Gu*

Department of Aquatic Animal Medicine, College of Fisheries, Huazhong Agricultural University, Wuhan, 430070, China. huruixue@webmail.hzau.edu.cn
zhangqi77@mail.hzau.edu.cn guzemao@mail.hzau.edu.cn

Frog farming, as a large proportion in aquaculture, has been practiced in many countries. The black-spotted frog *Pelophylax nigromaculatus*, endemic to East Asia, is one of the most widely farmed frogs in south-central China in the last five years. Since 2016, epidemic meningitis-like disease outbreaks in cultured black-spotted frogs occurred in separate farms.

To figure out the pathogenesis of this disease, a total of 213 abnormal black-spotted frogs were collected from seven separate farms in Hunan, China, during May to October 2016. After euthanasia, a routine necropsy and histopathology were performed. Bacteria isolation, microscopic parasites examination, PCR test for fungus and viruses were conducted for etiology detection. Histopathologic examination demonstrated chronic severe meningitis with denatured incrassated meninges. Bacterial infections (190/213) were confirmed in the etiological examination, and 90% of the isolates were identified as *Elizabethkingia miricola* according to 16S rRNA gene and gyrB gene. The pathogenicity of *E. miricola* was been verified by experimental challenges. *Elizabethkingia* was reported to be occasionally associated with human clinical infections with high mortality. Whole-genome sequencing revealed that this amphibian *E.miricola* is closely related to human clinical isolate, indicating that *E. miricola* can be epizootic and may pose a threat to humans. PFGE is ongoing to try to study more about the epidemiology of this pathogen.

We described infectious disease in amphibians caused by *Elizabethkingia* genus. As we known, *Elizabethkingia* has been reported to infect Chinese sturgeons (*Acipenser sinensis*) (Wei, 2018) and African catfish (*Clarias gariepinus*) (Laith, 2016). These studies indicate that *Elizabethkingia* is an emerging pathogen in aquatic animal, and the pathogenic mechanism needs to be further studied.

Conference Session Designation: (Flavobacteria)
Presentation Format: (Oral)
Student Presentation: (Yes)

Isolation, Identification and Characterization of a *Tenacibaculum dcentrarchi* Like Bacteria Causing Acute Disease and Mortality in Atlantic Salmon in a Norwegian Post Smolt Site.

Øystein Klakegg [1,2*], Takele Abayneh[3], Kira Salonius [1], Aud Kari Fauske [1,2], Henning Sørum [2]

[1] Previwo AS, Sofies Gate, Gate 88A, 0454 Oslo oystein.klakegg@previwo.no post@previwo.no
[2] Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Department of Food Safety and Infection Biology
[3] National Veterinary Institute, Bishoftu/Debre-zeit, Ethiopia

Particularly due to the challenges of sea lice, *Lepeophtheirus salmonis*, post smolt sites for aquaculture production of Atlantic salmon (*Salmo salar*) on land is desirable and the number of sites has grown significantly. At the post smolt sites the salmons can grow larger without exposure of sea lice and then make the exposure time for sea lice at sea shorter. However, it has been found that wounds and increased mortality is becoming a challenge at several post smolt sites.

This study reports an investigation of a case of disease outbreak at a Norwegian post smolt site, with 600 000 smolts divided in eight 750m^3 tanks, with the primary objective of isolation and characterization of the causative agent. A few days after transfer from freshwater to seawater at the post smolt site, the mortality at the site increased and soon the mortality was increased in all tanks. The salmon that died and found moribund had severe lesions, often 2-6 cm wide, particularly behind the pectoral fins. The lesions penetrate the skin as well as deep into the musculature. In some of the moribund salmon, the lesions were penetrating into the abdominal cavity and exposed internal organs as gut and liver which occasionally penetrate out of the wounds. We also saw fin rot, particularly on the pectoral fins. Culturing specimen taken from lesions on marine agar showed huge growth of one dominant bacteria colony. Microscopy showed rod shaped *Tenacibaculum* like bacteria. 16S rRNA showed that the dominant bacteria was a *Tenacibaculum dicentrarchi* like bacteria. Genetic characterization employing Multi Locus Sequence Analyze (MLSA) using seven housekeeping genes: atpA, dnaK, glyA, gyrB, infB, rlmN and tgt, was conducted. The MLSA analysis indicated that the isolates obtained in the outbreak belong to *T. dicentrarchi* where all were highly phylogenetically related to the *T. dicentrarchi* type strain (USC39/09^T) from Spain. *Tenacibaculum dicentrarchi* is known to be associated with tail roots, frayed fins and wounds in severe outbreaks in Atlantic salmon in Chile. In Norway as far as we know this is the first described outbreak of *Tenacibaculum dicentrarchi* in Atlantic salmon.

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

Natural Antibiotic Sensitivity and Biofilm Formation in *Flavobacterium*

Terence L. Marsh*[1], Marceline Stevens[1], Thomas Loch[2], Donna Ye[1] Mariane Mota Cavalcante[1], John Bauman[3], Roshan Angoshtari[1], Masanori Fujimoto[4] and Kim Scribner[5]

[1] Dept. of Microbiology and Mol. Genetics, Michigan State University, East Lansing, MI, USA marsht@msu.edu
[2] Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA
[3] Michigan Department of Natural Resources, Fisheries Division, Gladstone, Michigan
[4] Soil and Water Sciences Department, University of Florida, Gainesville, FL
[5] Department of Fisheries & Wildlife, Michigan State University, East Lansing, MI, USA

Our interest in *Flavobacterium* stems from investigations into the microbiome of sturgeon eggs and biofilm formation by environmental bacteria. Our interrogation of the sturgeon egg microbiome revealed that among the diverse phylogenetics of freshwater *Flavobacterium/Chryseobacterium*, a well-defined subset contributed to the egg microbiome. Moreover, treating hatchery eggs with formalin or peroxide shifted the community from 10-15% to as high as 82% *Flavobacterium*. Screening several hundred of isolates from the egg's microbiome identified six with antimicrobial activity, of which four had significant activity against fish pathogens *Aeromonas* spp., *Yersinia ruckeri* and *Flavobacterium* spp., as measured with a soft agar overlay test. We then tested the robustness of biofilm formed by these fish pathogens to challenge by our most aggressive antimicrobial-producing isolate, a *Pseudomonas* sp., and found that biofilm of one *Aeromonas* sp. was reduced but biofilm was elevated when *Flavobacterium* sp., *Yersinia ruckeri* and *F. columnare* were co-incubated with an antimicrobial-producing *Pseudomonas*.

In a separate line of investigation into environmental signals that trigger biofilm formation, we identified exogenous protein as a factor. Elevated protein concentrations in media were found to stimulate biofilm formation by *Serratia* spp., *Aeromonas* spp., and *Flavobacterium columnare*. A more detailed analysis of *Flavobacterium/Chryseobacterium* isolates from fish has identified 3 phenotypes in response to elevated protein. Under our laboratory conditions, some strains produce little biofilm, regardless of changes in nutritional conditions. A second phenotype produced reasonably robust biofilm under our standard conditions, but produced diminishing amounts of biofilm as the concentration of exogenous protein increased. Finally, *F. columnare* and *C. nakagawai* produce robust biofilms at an exogenous protein concentration of 1%. These increases in biofilm productivity as measured by crystal violet staining were accompanied by an increase in cells within the biofilm and an increase in protein in the biofilm matrix. *F. columnare* biofilm formed under elevated exogenous protein was robust and visualized with fluorescent microscopy, revealed a highly proteinaceous biofilm, indicating recruitment of exogenous protein from the media into the biofilm matrix.

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