# COMPARISON OF NORMAL GUT MICROFLORA OF WASHINGTON HATCHERY COHO SALMON (*ONCORHYNCHUS KITSUTCH*) TO PREVIOUSLY REPORTED GUT MICROFLORA OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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The purpose of this study is to determine if the predominant bacterial species found in the gut microflora of cultured juvenile Coho salmon (*Oncorhynchus kisutch*) are consistent with those previously reported in cultured rainbow trout (RBT) (*Oncorhynchus mykiss*). It is generally recognized that the intestinal flora of fish serves both in digestive functions and as a protective barrier against disease. From this study, we set out to gain a better understanding of gut flora in salmonids and an indication of how broadly results from such studies may be applied.

It has been reported that the dominant microflora in RBT intestines are culturable. Assuming that the non-pathogenic microflora have a disease-preventing effect, this protection is likely to be mediated by microorganisms that are present in high numbers. The predominant species found in RBT were *enterobacteracae, vibrionacae,* and *pseudomonads* (including *shewanella*). Gut flora from 20 coho was cultured on brain heart infusion agar (BHIA), and predominant colony types were selected, isolated, and characterized. Microscopy and biochemical methods were used to determine if the selected bacteria fell into the above-mentioned classifications. The analysis of gut flora showed that coho salmon, as had been previously reported in rainbow trout, had similar microflora with the predominant groups being variable in both species. While there were no clear candidates for probiotics selected as a result of this research, a better understanding of how to proceed in the investigations of characterizing normal gut flora was developed.

## INTRODUCTION

Fisheries are part of the Pacific Northwest culture. Because of habitat loss and the impacts of an increasing human population, the ability of fish to naturally sustain their populations is impaired. Hatcheries are important as a tool to rebuild wild populations and sustain fisheries as habitat needs and other impacts are addressed. Within hatcheries, fish health pathologists monitor and maintain the health of the hatchery fish. There are many approaches to maintaining the health of hatchery fish, including probiotics, which is a way of raising resistance to pathogens by administering selected helpful bacteria that will survive in the gastrointestinal tract. Studies have shown increased resistance to fish pathogens as well as enhanced health in finfish when probiotics are used. If probiotics are to be effectively developed for coho salmon (*oncorhynchus kitsutch*), an understanding of normal gut microflora needs to be established so that appropriate species of bacteria can be selected. This project was designed to investigate the gut microflora in coho salmon.

Spanggaard et al[1] reported a high degree of variability between the bacteria cultured from intestinal tracts of rainbow trout (*oncorhynchus mykiss*). This current study was designed to see if the same degree of variability would be observed in coho salmon from two western Washington hatcheries: Kalama Creek hatchery and Skookum Creek hatchery. By using some of the methods reported by Spanggaard et al,[1] our study compared their results to coho salmon. The hypothesis was that the gut microflora in coho salmon would be as variable as reported in cultured rainbow trout.

## METHODS

The intestinal tracts were removed from 10 juvenile fish from Kalama Creek hatchery and 10 juvenile fish from Skookum Creek hatchery. All intestinal tracts were removed using aseptic technique and all fish had been fed before intestinal tracts taken. Each intestinal tract was diluted in sterile PBS (phosphate buffered saline) and spread on BHIA in .3 and .1 milliliter aliquots. The plates were grown for at least 48 hours before dominant colonies on plates were re-struck onto new plates and grown again. The other bacterial growths (non-dominant) were observed and recorded but otherwise no longer observed. Twenty-one colonies were isolated and re-struck from Kalama Creek while nine colonies were isolated and re-struck from Skookum Creek. Biochemical tests (including gram-stain, motility, oxidase, catalase, and glucose utilization) were run on all isolates. The isolates were then grouped by their test results and keyed against the bacterial groups that Spanggaard et al[1] had reported. The bacteria groups included: *enterobacteriaceae, acinetobacter/moraxella, vibrionaceae, pseudomonas spp./Shewanella,* other Gram-negatives, and Gram-positives.

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**Table 1. Results from the 21 Kalama Creek isolates**

| | Kalama Creek Hatchery | | | | | |
|---|---|---|---|---|---|---|
| **Isolate #** | **Gram stain** | **Cata-lase** | **Motility** | **Oxi-dase** | **Glucose oxid/ ferm.** | **Category** |
| 4a | − | − | − | − | Ng | other |
| 1a | − | − | + | − | O | other |
| 1c.1 | − | − | + | + | Nc | other |
| 5a | − | − | + | + | F | other |
| 7a | − | − | − | + | Nc | other |
| 7b, 8a | − | + | + | + | Ng | other |
| 3a, 6c, 6d, 8b, 9a | − | + | − | − | Nc | *Acinetobacter/Moraxella* |
| 4b, 9b | − | + | − | + | Nc | *Acinetobacter/Moraxella* |
| 10a | − | + | + | − | F | *Enterbactericeae* |
| 2b, 6b, 10b | − | + | + | + | Fg | *Vibrioaceae* |
| 1c, 2 | − | + | + | + | Nc | *Pseudomonas/Shewanella* |
| 1b | + | + | − | − | Nc | gram + |
| 2a | + | + | + | − | Nc | gram + |

o = oxidase; f = fermintative; fg = fermintative with gas; nc = no change; ng = no growth.

## DATA ANALYSIS

Table 1 shows the test results from the 21 Kalama Creek isolates that were characterized. They are grouped according to test results, with the final classification in the last column. Table 2 shows similar results for Skookum Creek. We also saw variability between the numbers and types of bacteria initially isolated from the fish from each of the hatcheries, although this was not quantified.

## RESULTS

There were 21 isolated colonies from Kalama Creek, and 9 isolated colonies from Skookum Creek. The Kalama Creek isolates fell into all of the six groups while Skookum Creek isolates had only 3 groups represented, with half of them occurring in one group (pseudomonas/*shewanella*). With the small size of this study, it is recognized that an increased number of isolates could change these patterns.

In Spanggaard's Farm 1 group, the predominant group characterized was *entrobacteriaceae.* In that study's Farm 2 group, there were no *entrobacteriaceae* and the predominant group was other Gram-negatives. In Farm 3 group, there were no other Gram-negatives and the predominant group was *Vibrionacceae.* When the same farm was tested again 3 months later, there were some *vibrionacceae* but the predominant group was now *enterobacteriaceae.* In our study of the Kalama group, the predominant group was split between *acinetobacter/moraxella* and other Gram-negatives. The predominant group in Skookum was *pseudomonas/shewanella.*

**Table 2. Results from the 21 Shookum Creek isolates**

| | Shookum Creek Hatchery | | | | | |
|---|---|---|---|---|---|---|
| **Isolate #** | **Gram stain** | **Cata-lase** | **Motility** | **Oxi-dase** | **Glucose oxid/ ferm.** | **Category** |
| 5a, 8a | − | − | + | + | Fg | other |
| 6b | − | − | − | − | Nc | other |
| 3a | − | + | − | − | Fg | *Enterobactericeae* |
| 1a, 4a, 7a, 7b, 10a | − | + | + | + | Nc | *Pseudomonas/Shewanella* |

o = oxidase; f = fermintative; fg = fermintative with gas; nc = no change; ng = no growth.

## CONCLUSION

Culturing bacteria on BHIA was an effective method for isolating gut microflora. We successfully characterized the organisms that we isolated from the intestine of juvenile salmon.

We did not detect one consistent predominant group of bacteria between the sample groups, and there was no common pattern of microflora. Therefore the hypothesis that the microflora in coho salmon would be as variable as in rainbow trout was supported by these results.

The variability in gut microflora could be caused by any number of factors including feed, water, temperature, environment, and living conditions. These results suggest that future studies should be done to better understand the normal gut microflora in coho salmon. As probiotics are developed, recognition of this variability will be important to its success.

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### REFERENCE

1. Spanggaard B, Huber I, Nielsen J, Nielsen T, Appel KF, Gram L. The microflora of rainbow trout intestine: a comparison of traditional and molecular identification. *Aquaculture.* 2000; 182:1–15.