Report for 2002AK7B: Luminescent Bacteria: A New Water Quality Issue?

- Articles in Refereed Scientific Journals:
  - Budsberg, K.J., C.F. Wimpee and J.F. Braddock. Isolation and identification of Photobacterium phosphoreum from an unexpected niche: migrating salmon. In Review. Applied and Environmental Microbiology.
- Conference Proceedings:
  - Budsberg, K.J., C.F. Wimpee and J.F. Braddock. 2003. Phenotypic characterization of Photobacterium phosphoreum from migrating Alaskan salmon. In Abstracts of the 103th General Meeting of the American Society for Microbiology, Washington, DC, May 2003.
  - Budsberg, K.J., C.F. Wimpee and J.F. Braddock. 2003. Genomic cloning of the lux operon from luminous Alaskan isolates. Oral presentation at the Alaska Chapter of the American Society for Microbiology 19th Annual Meeting, Anchorage, AK, April 2003.
  - Budsberg, K.J., C.F. Wimpee and J.F. Braddock. 2002. Isolation and identification of Photobacterium phosphoreum from a new niche: Yukon River salmon, Alaska, USA. In Abstracts of the 102th General Meeting of the American Society for Microbiology, Salt Lake City, UT, May 2002, p. 248.

Report Follows:

Problem and Research Objectives

The overall objective of this study is to characterize luminescent bacteria isolated from salmon harvested for subsistence use on the Yukon River. Subsistence fishers from several native villages on the Yukon River reported fish in fall 2001 on fish racks "glowing in the dark" This caused substantial alarm in the rural community as an important and traditional food source appeared to be tainted. It is not clear why this phenomenon has not been more widespread in other years, although fishers on the Yukon River have noted limited occurrences for a number of years (Randy Brown, U.S. Fish and Wildlife Service, personal communication). It is also not known how commonly luminescent bacteria are found on salmon returning to spawn from the marine environment. Furthermore, until this study, it was not known exactly what species of bacteria were responsible for the outbreak seen in 2001.

It is important to understand that no known luminescent bacteria are pathogenic but several species are closely related to known human and fish pathogens (e.g., several strains of *Vibrio cholerae* and *V. vulnificus* are bioluminescent). In addition, the presence of luminescent bacteria on fish in the cold smoke processes has been used as an indicator of spoilage. Our preliminary data indicated that luminescent isolates from Alaskan salmon may be different from other known luminescent bacteria. Thus to assure the safety of fish on which these bacteria are growing, the identity of the bacteria was very important to determine.

Subsistence fishers on the Yukon River have observed "glowing" fish in other years (Randy Brown, U.S. Fish and Wildlife Service, personal communication), but the phenomenon was widespread in fall 2001. It is known that luminescent bacteria "light up" only upon reaching a high population density; apparently conditions favored growth of these bacteria in summer 2001. The presence of luminescence on Yukon River salmon led to a number of interesting questions: (1) What taxonomic groups do our bacterial isolates belong to? (2) Are the Alaskan isolates taxonomically different from other previously described luminescent bacteria? (3) How are the bacteria transported to the freshwater environment? Via the fish gut or slime layer or other parts of the fish? (4) Are other species of luminescent bacteria present in fish traveling up Alaskan rivers to spawn? (5) Can planktonic luminescent bacteria be found in the Yukon River? (6) Assuming our isolates are of marine origin, do the bacteria possess physiological adaptations that allow them to survive in the freshwater habitat? (7) Were conditions unique in summer 2001 that allowed more widespread occurrence of luminescence on fish? (8) Are the isolate from 1997 and the isolates from 2001 the same organism? (9) Are isolates found in different locations on a single fish the same organism? (10) Is the occurrence of "glowing fish" likely to be high in the future due to other factors such as global change or the overall health of the fish? The first year of this project focused on answering questions (1), (2), (4), (6) and (8).

Approaches and Methodology:

To characterize our isolates first extensive phenotypic analyses were performed (as described in Reichelt and Baumann, 1973; Nealson, 1978). Second, characterization of the *luxA* genes were performed as previously described (Wimpee et al., 1991). Briefly, genomic DNA was isolated, *luxA* primers were used in PCR amplification, amplified fragments were cloned into pCRII-TOPO (Invitrogen Corp.), and the resultant cloned DNA was sequenced on an automated sequencer. The sequences were subjected to phylogenetic analysis using programs in the PAUP package (Swofford, 1998). Thirdly, a similar strategy was used for the 16S rRNA

gene. Universal bacterial primers ("11F " and "1492R") were used to amplify 16S, followed by cloning, sequencing, and phylogenetic analysis. Finally, in addition to the sequence analysis of *luxA* and 16S, a genomic library was constructed, and the cloned *luxA* gene was used as a probe to isolate the entire *lux* operon from our Alaskan strains.

Principle Findings and Significance:

All our Alaskan isolates are short rods, oxidase negative, Gram negative, and require L-methionine for growth in minimal media. Additionally, all Alaskan isolates grow at 4° C, however, optimal growth occurred at 10 - 15° C; no growth occurred at or above 20° C. Growth required the presence of sodium chloride in the medium and cells did not remain viable in river water unless amended with sodium chloride. Comparing our nutritional versatility data to published references, we can place all Alaska strains in the *P. phosphoreum* group. To verify our results, a reference strain, *P. phosphoreum* NZ-11-D, was included in the test (Table 1). The ability of NZ-11-D to utilize acetate and AK-3 to utilize pyruvate do not present any difficulty in placing Alaskan strains in the *P. phosphoreum* group because of the genetic information described below.

SSU rDNA gene sequences of the seven AK isolates were aligned with six representative sequences from other luminous bacteria. The alignment produced a consensus sequence 1,159 bp in length shared by all 13 taxa. Maximum likelihood analysis of the alignment by PAUP v4.0 revealed all AK isolates cluster identically with *P. phosphoreum* (Figure 1). *E. coli* was used as the outgroup in the maximum likelihood analysis of the SSU rDNA genes.

luxA (a gene necessary for luminescence in all luminescent bacteria) sequences of the seven AK isolates were aligned with six representative sequences from other luminous bacteria. The alignment produced a consensus sequence 554 bp in length shared by all 13 taxa. Maximum likelihood analysis of the alignment by PAUP v4.0 revealed all AK isolates cluster closely with *P. phosphoreum* (Figure 2). *V. harveyi luxB* was used as the outgroup in the maximum likelihood analysis of the *luxA* genes.

Our Alaskan strains of *P. phosphoreum* are nearly identical to other descriptions with respect to nutritional versatility, *luxA* and SSU rDNA sequences; however, our isolates appear to have a lower optimal growth temperature as compared to the reference strain, *P. phosphoreum* NZ-11-D. Future investigations of the osmotic requirements and temperature tolerances of Alaskan *P. phosphoreum* may reveal adaptations specific to this unique niche. In year two of the study we will continue the genetic analyses of our isolates and begin to address ecological questions related to where the bacteria are located on migrating salmon and how prevalent the occurrence is of luminous bacteria on migrating salmon.

References

Nealson, K.H. 1978. Isolation, identification and manipulation of luminous bacteria. Methods Enzym. 57: 153-156.

Reichelt, J.L. and P. Baumann. 1973. Taxonomy of the marine, luminous bacteria. Arch. Mikrobiol. 94: 283-330.

Swofford, D.L. 1998. Phylogenetic Analysis Using Parsimony. Version 4.0.

Wimpee, C.F., T.-L. Nadeau and K.H. Nealson. 1991. Development of species-specific hybridization probes for marine luminous bacteria by using in vitro DNA amplification. Appl. Environ. Microbiol. 57: 1319-1324.

Table 1. Phenotypic characteristics of our isolates.

	Published Reference Data					Tested Strains						
	V. harveyi [a]	*V. fischeri* [a]	*P. leiognathi* [a]	*P. phosphoreum* [a,b]	*P. phosphoreum* NZ-11-D[c]	NZ-11-D	AK-1	AK-5	AK-6	AK-7	AK-8	AK-9
Growth on:												
Maltose (0.2%)	+	+	-	+	+	+	+	+	+	+	+	+
Cellobiose (0.2%)	+	+	-	-	-	-	-	-	-	-	-	-
Glucuronate (0.1%)	+	-	-	(+)	(+)	+	-	-	-	-	-	-
Mannitol (0.1%)	+	+	-	-	(-)	-	-	-	-	-	-	-
Proline (0.1%)	+	+	+	(-)	(-)	-	-	-	-	-	-	-
Lactate (0.2%)	+	-	+	(-)	-	-	-	-	-	-	-	-
Pyruvate (0.1%)	+	-	+	-	-	-	-	-	-	-	-	-
Acetate (0.05%)	+	-	+	-	-	-	-	-	-	-	-	-
Propionate (0.05%)	+	-	-	-	-	-	-	-	-	-	-	-
Heptanoate (0.05%)	+	-	-	-	-	-	-	-	-	-	-	-
D-α-Alanine(0.05%)	+	(-)	-	-	-	-	-	-	-	-	-	-
L-tyrosine (0.4%)	+	-	-	-	-	-	-	-	-	-	-	-
Production of:												
Lipase	+	-	-	-	-	-	-	-	-	-	-	-
Gelatinase	+	-	-	-	-	-	-	-	-	-	-	-
Amylase	+	-	-	-	-	-	-	-	-	-	-	-
Optimal growth temperature:					20° C	22° C	15° C	15° C	15° C	15° C	15° C	15° C

Parantheses in the published reference data indicates strain variability. Tested strains are isolates from Alaskan salmon. [a]Taxonomic information from Nealson (1978), [b]Bergey's Manual of Diagnostic Bacteriology (1994), and [c]Nealson (1993).

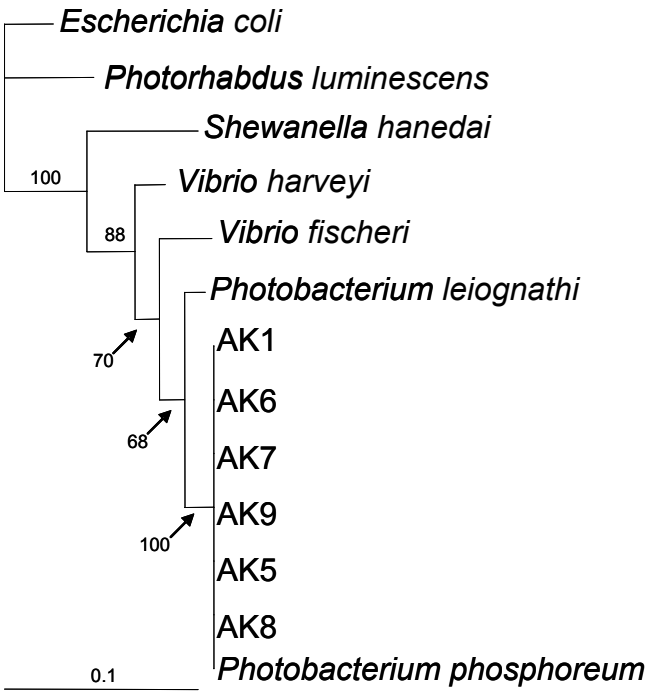

Fig. 1. Phylogeny of Alaskan luminous bacteria based on Maximum Likelihood analysis using PAUP* 4.0b10 with SSU rDNA sequences from Alaskan isolates and representative sequences from GenBank. All strains with "AK" are from salmon harvested from the Yukon River, Alaska. *E. coli* was used as the outgroup in this analysis.

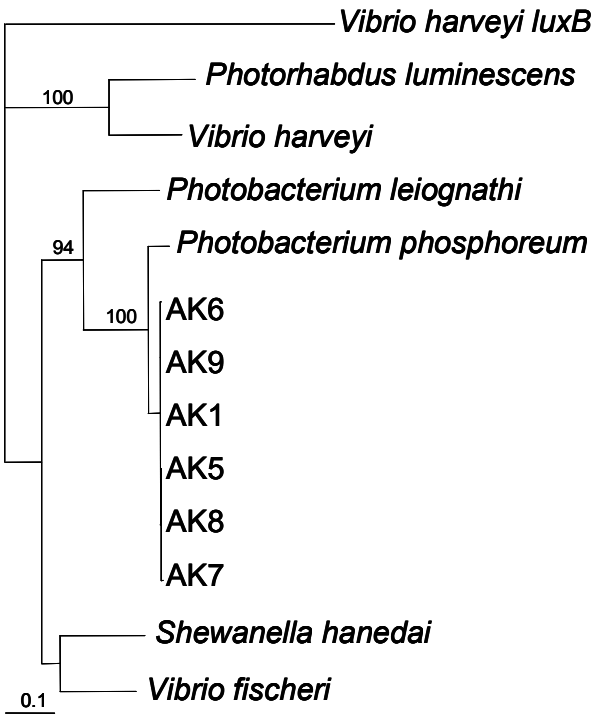

Fig. 2. Phylogeny of Alaskan luminous bacteria based on Maximum Liklihood analysis using PAUP* 4.0b10 with *luxA* sequences. All strains with "AK" are from salmon harvested from the Yukon River, Alaska. *V. harveyi luxB* was used as the outgroup in the Maximum Likelihood analysis of *luxA* genes.