



## Information bulletin

U.S. DEPARTMENT OF THE INTERIOR  
NATIONAL BIOLOGICAL SURVEY

NO. 36  
DATE 1994

# Media Composition Has Little Effect on Growth of Two Fish Pathogens, *Cytophaga columnaris* and *Cytophaga psychrophila*

*Cytophaga columnaris* (= *Flexibacter columnaris*) and *Cytophaga psychrophila* are two yellow-pigmenting species of bacteria often associated with disease in fish. This study compared growth of both pathogens in media used by fish health biologists to culture yellow-pigmenting bacteria. All bacteria used in this study (ten isolates per species) were originally obtained from diseased fish. *Cytophaga columnaris* causes Columnaris disease and induces pathology in both warmwater and coldwater fishes. *Cytophaga psychrophila* causes cold-water disease and this condition is principally a problem for salmonid fishes. Both bacteria require isolation on plating media with reduced agar and nutrient concentrations, but growth is limited. We did this study to see if growth could be increased to improve diagnostic methods.

### Bacterial Cultivation

Bacteria were originally inoculated onto *Cytophaga* agar for 24 h to provide initial inocula for further study. *Cytophaga columnaris* was incubated at 20°C and *C. psychrophila* was incubated at 15°C throughout the study. After incubation, growth was washed from the agar with sterile, phosphate-buffered saline (pH = 7.2) and each bacterial suspension was standardized with phosphate buffer to a 60% transmittance (525 nm) using a

Spectronic-20 colorimeter. Aliquots (0.1 mL) of standardized suspensions were used to inoculate 5.0 mL of each broth medium described in Table 1. Cultures were incubated for 48 h and were quantified on an agar-based form of the respective medium in which they were incubated by dilution drop counts.

### Bacterial Growth Was Not Influenced by Medium Composition

Our results indicated that growth of either *C. columnaris* or *C. psychrophila* was not selectively enhanced by culture in the seven media that were tested (Table 2). Although bacterial concentrations for *C. psychrophila* were somewhat higher in Medium A (Table 2), differences in the culture of either pathogen may simply reflect the individual preference of a given diagnostician rather than specific nutritional effects on the bacteria.

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**Table 1.** Composition and source of media used to culture yellow-pigmenting bacteria.

Ingredient	Grams of ingredient per 100 mL of medium						
	A <sup>1</sup>	B <sup>2</sup>	C <sup>3</sup>	D <sup>4</sup>	E <sup>5</sup>	F <sup>6</sup>	G <sup>7</sup>
Yeast extract	0.05	0.20	0.05	0.05	0.04	0.04	0.05
Beef extract	0.02	0.05					
Tryptone 0.05			0.20	0.40	0.40	0.20	
Casitone		0.05	0.30				
Gelatin							0.20
Glucose						0.05	
Calcium chloride		0.02	0.03		0.05		
Magnesium sulfate						0.05	
Sodium acetate	0.02	0.02					

<sup>1</sup>Anacker, R.L., and E.J. Ordal. 1959. Studies on the myxobacterium *Chondroccus columnaris*. 1. Serological typing Journal of Bacteriology 78:25-32.

<sup>2</sup>Anderson, J.I.W., and D.A. Conroy. 1969. The pathogenic myxobacteria with special reference to fish diseases. Journal of Applied Bacteriology 32:30-39.

<sup>3</sup>Bootsma, R., and J.P.M. Clerx. 1976. Columnaris disease of cultured fish *Cyprinus carpio* L. Characteristics of the causative agent. Aquaculture 7:371-384.

<sup>4</sup>Hsu, T-C., E.B. Shotts, and W.D. Waltman. 1983. A selective medium for the isolation of yellow pigmented bacterias associated with fish disease. Newsletter for the Flavobacterium-Cytophaga Group 3:29-30.

<sup>5</sup>Fujihara, M.P. and R.E. Nakatani. 1971. Antibody production and immune responses of rainbow trout and coho salmon to *Chondroccus columnaris*. Journal of the Fisheries Research Board of Canada 28(9):1253-1258.

<sup>6</sup>Holt, R.A. 1987. *Cytophaga psychrophila*, the causative agent of bacterial cold-water disease in salmonid fish. Ph.D. Thesis. Oregon State University. Corvallis, OR. 181 pp.

<sup>7</sup>Bullock et al. (1986). Columnaris disease of fishes. Fish Disease Leaflet #72. Fish and Wildlife Service, Washington, D.C. 9 pp.

**Table 2.** Growth of *Cytophaga columnaris* and *Cytophaga psychrophila* after 48 h in different media. Growth is designated as the mean of 10 isolates per species.

	A*	B	C	D	E	F	G
<i>Cytophaga columnaris</i> :							
Mean	4.0×10 <sup>6a</sup>	7.3×10 <sup>5a</sup>	6.3×10 <sup>6</sup>	8.1×10 <sup>6</sup>	2.6×10 <sup>7</sup>	2.2×10 <sup>7</sup>	1.9×10 <sup>7</sup>
<i>Cytophaga psychrophila</i> :							
Mean	5.1×10 <sup>7b</sup>	1.3×10 <sup>7</sup>	1.2×10 <sup>7</sup>	7.5×10 <sup>6</sup>	8.0×10 <sup>6</sup>	1.1×10 <sup>7</sup>	2.4×10 <sup>6</sup>

\* Designates medium as described in Table 1.

<sup>a</sup>Statistical difference between growth in media A and B, Least Significant Difference of the Means Test ( $P < 0.05$ ,  $LSD = 3.6 \times 10^7$ ). No difference was noted amongst and between comparisons of any other means.

<sup>b</sup>Denotes statistical difference between growth in medium A and all other groups, ( $P < 0.05$ ,  $LSD = 3.4 \times 10^7$ )