APPLIED MICROBIOLOGY, Jan., 1965 Vol. 13, No. 1
 Printed in U.S.A.

Characteristics and Pathogenicity of a Capsulated *Pseudomonas* Isolated from Goldfish

GRAHAM L. BULLOCK

Bureau of Sport Fisheries and Wildlife, Eastern Fish Disease Laboratory, Kearneysville, West Virginia

Received for publication 24 August 1964

ABSTRACT

BULLOCK, GRAHAM L. (Bureau of Sport Fisheries and Wildlife, Kearneysville, W. Va.). Characteristics and pathogenicity of a capsulated *Pseudomonas* isolated from goldfish. Appl. Microbiol. **13**:89-92. 1965—Characteristics of a capsulated bacterium isolated from an epizootic among goldfish (*Carassius auratus*) were determined as well as the ability of the bacterium to produce experimental infections. The bacterium was found to be a gram-negative rod which oxidized carbohydrates, produced green fluorescent pigment, and otherwise seemed to fit into the genus *Pseudomonas*, except that it was nonmotile and failed to oxidize gluconate. These last two characteristics are typical of pseudomonads. However, the bacterium was classified as a pseudomonad, best fitting the description of the nonmotile variety of *Pseudomonas fluorescens*. Goldfish and rainbow trout (*Salmo gairdneri*) were infected experimentally by injection, but not by being fed bacteria-laden food. Goldfish were infected by exposure to the bacterium, but only if two or three scales were removed prior to exposure. It is suggested that the bacterium is an opportunistic pathogen.

On two occasions in January 1963, moribund goldfish (*Carassius auratus*) involved in an epizootic in a private hatchery were brought to this laboratory for examination. The most noticeable gross symptoms were listlessness, and areas of hemorrhages in the fins, body wall, and viscera. Some fish also had an accumulation of bloody, peritoneal fluid indicating a severe bacteriemia. All fish examined contained large numbers of gram-negative bacteria in the kidney and peritoneal fluid. A halo of faintly staining capsular material surrounded many of the cells in smears prepared from fish tissues.

Two types of gram-negative bacteria were isolated. A nonmotile rod, encapsulated in the fish and producing abundant slime on agar media, was isolated in both cases; a motile bacterium identified as *Aeromonas liquefaciens*, a known pathogen of warmwater fish, was isolated in the first examination only. Because the encapsulated nonmotile bacterium was not a recognized fish pathogen and was isolated from fish on both occasions, a further study of its characteristics and pathogenicity was undertaken.

EXPERIMENTAL METHODS AND RESULTS

Description of isolates. Three isolates were examined and found morphologically and physiologically identical. All cultures were incubated at 20 C. Cells from 24-hr Trypticase Soy Agar (TSA, BBL) slant cultures were gram-negative, occurred singly and in pairs, and had an average length of 1.8 μ and an average width of 0.73 μ. Cells from infected fish were of almost equal size. Flagella could not be detected by use of the method of Novel (1939). Spores were never observed in any cultures, and the bacterium failed to survive at 65 C for 30 min. The growth on agar-slant cultures was viscid, because of the abundant slime produced. Colonies were round with an entire edge, markedly convex, and also slimy (Fig. 1).

Discrete capsules, staining lightly with carbol fuchsin or Gram stain, were consistently observed from infected fish (Fig. 2); however, the appearance of the capsules was not consistent. In experimental infections, the capsules appeared distinct and sharply outlined when material from the first mortality was examined. However, as the experimental infection progressed, they appeared to lose distinctness and were banded, apparently due to selective staining in some areas. As determined by the staining technique of McKinny (1953), the capsular material contained polysaccharide; with the Giemsa stain, the material gave an acid reaction.

While capsules could be seen around some cells grown on nutrient agar (Difco), TSA, or TSA

enriched with beef serum (especially with the flagella stain), they were not a consistent feature. However, an amorphous slime layer was present on all agar media tried, regardless of whether the medium had additional carbohydrate. The slime layer showed the same, but weaker, reactions with the Giemsa stain and the carbohydrate stain of McKinny (1953). Additional characteristics of the bacterium are given in Table 1. The carbohydrate reactions appeared much sooner and were easier to interpret when O-F Basal Medium (Difco) was used than when the peptone base was employed. The oxidative nature of the bacterium was also more readily observed in the O-F Medium containing carbohydrate. The cytochrome oxidase test (Ewing and Johnson, 1960) was more readily demonstrated on 24- to 48-hr agar-plate cultures than on slant cultures the same age. Also, although green fluorescent pigment was produced on *Pseudomonas* F Agar (Difco) after 24 hr, it was contained primarily in the growth area, and slowly leached into the surrounding medium after 72 to 96 hr. It is possible that the slime produced offered a physical barrier to the chemicals used in the cytochrome oxidase test and to the diffusion of the fluorescent pigment.

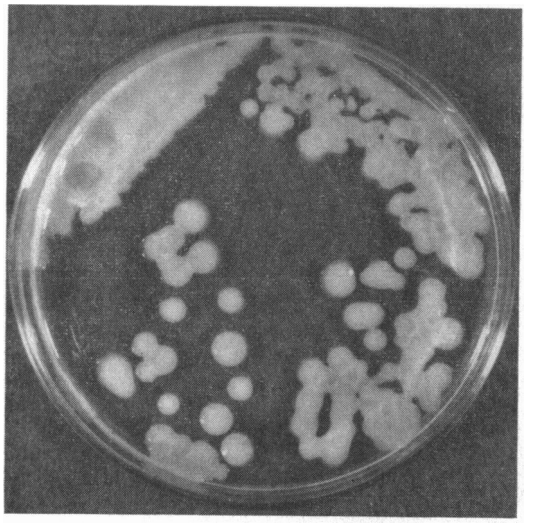

FIG. 1. *Plate culture (48-hr) of the nonmotile pseudomonad isolated from goldfish.*

Pathogenicity. Attempts were made to infect adult goldfish (8 to 13 cm long) and rainbow trout (*Salmo gairdneri;* 10 to 15 cm long) by injections and feedings with the bacterium. Goldfish were also challenged by addition of bacteria to the water.

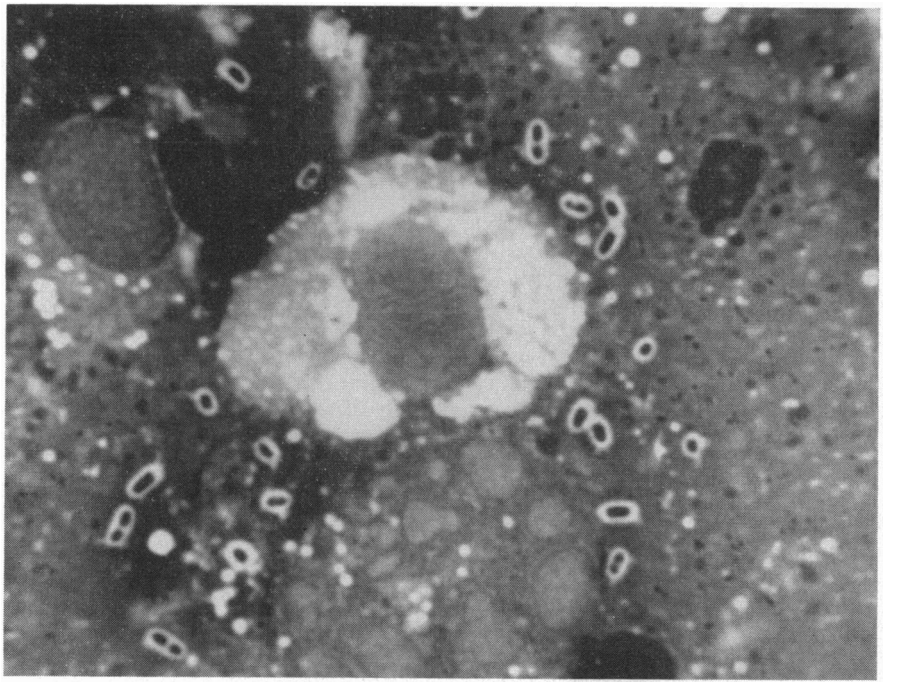

FIG. 2. *Smear taken directly from kidney and peritoneal fluid of infected goldfish showing the capsulated nonmotile pseudomonad. Magnification, 1,500 ×.*

TABLE 1. *Characteristics of nonmotile pseudomonad from goldfish*

Test	Result
Reduction of nitrate (nutrient broth plus 0.1% potassium nitrate)	+ (1)*
Disappearance of nitrite (nutrient broth plus 0.1% potassium nitrate)	+ (3)
H_2S in motility sulfide medium	− (7)
Indole (1% Tryptone broth)	− (7)
Growth in Koser Citrate Medium	+ (1)
Methyl red (Mr-VP broth)	− (7)
Voges-Proskauer (Mr-VP broth)	− (2, 7)
Amylase (nutrient agar plus 0.2% soluble starch)	− (7)
Gelatinase (nutrient agar + 0.4% gelatin)	+ (2)
Green fluorescent pigment in *Pseudomonas* F Agar	+ (2, 3)
Cytochrome oxidase	+ (1, 2)
Relation to free oxygen (agar shake culture)	Strict aerobe
Growth at 2 C	+ (4)
Growth at 37 C	− (7, 3)†
Oxidation of gluconate (method of Haynes, 1951)	− (7)
Acid from carbohydrates (nutrient broth base)	
Dextrose	+ (3)
Sucrose	− (14)
Mannitol	− (14)
Maltose	− (14)
Lactose	− (14)
Acid from carbohydrates (O-F base, open tube only)	
Dextrose	+ (1)
Sucrose	+ (7)
Mannitol	− (14)
Maltose	− (14)
Lactose	− (14)

* Numbers in parenthesis indicate day that test was positive or negative.

† Transfer from original 37 C culture; also failed to grow.

For the injection studies, cells were scraped from the surface of TSA slants, suspended in sterile saline, and further diluted (1:10) with sterile saline. Test fish were injected intramuscularly or intraperitoneally with 0.2 ml of either original or diluted cell suspension. Viable cell counts were made on an original cell suspension by use of the indirect poured plate method given in the *Manual of Microbiological Methods* (Society of American Bacteriologists, 1957), but with 99 ± 1 ml of saline blanks, and by plating all dilutions in duplicate. Similar suspensions were then prepared for use.

Heavily seeded commercial pelleted food was fed for 4 consecutive days to another group of goldfish and rainbow trout.

Attempts were made to infect goldfish held in aquariums by adding 6 ml of a 24-hr broth culture for 3 days. Goldfish in one aquarium were mechanically injured by removing two to three scales before addition of bacteria. Spring water of known composition (Warren, 1963) and a constant temperature of 12.5 C was used in all experiments.

Mortality caused by the test organism occurred only in those groups which received injection of cells, or where the fish were mechanically injured prior to exposure to the bacteria (Table 2). As judged by results with the 1:10 dilution, the bacterium was more pathogenic to the goldfish than to trout.

The bacterium was readily isolated in pure culture on *Pseudomonas* F Agar from internal organs of injected and also mechanically injured and exposed fish. Experimentally infected goldfish displayed essentially the same symptoms as seen in the original epizootic, except for more pronounced hemorrhaging around the mouth and gill cover. Trout also showed similar symptoms, and in addition, also had a thick yellow fluid present in the intestine.

DISCUSSION

The oxidative carbohydrate metabolism, production of cytochrome oxidase, fluorescence, and other properties (Table 1) would indicate that this bacterium belongs in the genus *Pseudomonas*. The presence of extracellular slime is also a common feature of the genus (Rhodes, 1958, 1959; Lysenko, 1961), but capsule formation in pseudomonads, according to Rhodes (1958), can depend on age of culture and medium used. This may explain why capsules were only seen consistently in smears of material from infected fish, and became less distinct as the infection progressed. Two features common to most pseudomonads, motility and oxidation of gluconate, are lacking in the bacterium described. Nevertheless, with the characteristics determined, the bacterium seems to fit the description of the nonmotile variety of *Pseudomonas fluorescens* as given in *Bergey's Manual*.

Epizootics among fishes involving aquatic pseudomonads are reported occasionally; the most recent is a disease outbreak in white catfish (*Italurus catus*) (Meyer and Collar, 1964). Relatively few disease outbreaks occur even though the bacteria are present in the water; thus, these

TABLE 2. *Effects of nonmotile pseudomonad on goldfish and rainbow trout*

Fish	Mode of challenge				
	Parenteral*		Enteral†	Adding cells to water†	
	Original suspension (6.4×10^7 cells injected)	1:10 dilution (6.4×10^6 cells injected)		Scales removed	Scales not removed
Goldfish (*Carassius auratus*)	17/18‡	16/18	0/10	7/10	0/10
Rainbow trout (*Salmo gairdneri*)	15/18	5/18	0/10	Not used	Not used

* Results obtained in 4 to 8 days.
† Results obtained in 21 days.
‡ Results expressed as number of dead fish per total number of fish.

pseudomonads are probably opportunists which produce disease when fish are stressed or injured. Also, because capsules are associated with virulence in some pathogenic bacteria, it is possible that the presence of a capsule in the present bacterium contributed to its pathogenic nature.

LITERATURE CITED

EWING, W. H., AND J. G. JOHNSON. 1960. The differentiation of *Aeromonas* and C27 cultures from *Enterobacteriaceae*. Intern. Bull. Bacteriol. Nomencl. Taxonom. **10**:223–230.

HAYNES, W. C. 1951. *Pseudomonas aeruginosa*—its characterization and identification. J. Gen. Microbiol. **5**:939–950.

LYSENKO, O. 1961. *Pseudomonas*—an attempt at a general classification. J. Gen. Microbiol. **25**:379–408.

MCKINNY, R. E., 1953. Staining bacterial polysaccharides. J. Bacteriol. **66**:453–454.

MEYER, F. P., AND J. D. COLLAR. 1964. Description and treatment of a *Pseudomonas* infection in white catfish. Appl. Microbiol. **12**:201–203.

NOVEL, E. 1939. Une technique facile et rapide de mise en evidence des cils bacteriens. Ann. Inst. Pasteur **63**:302–311.

RHODES, M. E. 1958. The cytology of *Pseudomonas* spp. as revealed by a silver-plating method. J. Gen. Microbiol. **18**:639–648.

RHODES, M. E. 1959. The characterization of *Pseudomonas fluorescens*. J. Gen. Microbiol. **21**: 221–268.

SOCIETY OF AMERICAN BACTERIOLOGISTS. 1957. Manual of microbiological methods. McGraw-Hill Book Co., Inc., New York.

WARREN, J. W. 1963. Kidney disease of salmonid fishes and the analysis of hatchery waters. Progressive Fish Culturist **25**:121–131.