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A UNIQUE BACTERIUM PATHOGENIC FOR WARM-BLOODED AND COLD-BLOODED ANIMALS

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The vast majority of bacterial fish diseases are caused by motile or nonmotile Gram-negative bacteria. Some of these, such as *Bacterium salmonicida*, have stable characteristics and represent bacterial species with well-defined properties. There are, however, many inadequately described motile Gram-negative bacteria which have been isolated from diseased warm- and cold-water fishes, amphibians, and reptiles from all over the world. Many of these bacteria belong to the genus *Pseudomonas*. Some bacteria, pathogenic to amphibians and reptiles (Hinshaw and McNeil 1946, 1946a, 1947), have been recently described and classified as paracolons.

The microorganism described in this report has a peculiar taxonomic position, because some of its characteristics indicate that it should be classified as a pseudomonad, while its physiological and antigenic properties suggest relationship with the paracolons.

Paracolon organisms isolated from outbreaks of gastrointestinal diseases in man have been described as a group of aberrant coliform organisms comprising a distinct biological group (Stuart et al. 1943). Borman, Stuart, and Wheeler (1944) referred coliform-like bacteria that slowly fermented lactose to a separate genus, *Paracolobactrum*. Those organisms which produced acetylmethylcarbinol were termed *Paracolobactrum aerogenoides*.

This report is believed to be the first description of organisms conforming largely to the description of *Paracolobactrum aerogenoides* and pathogenic for fish. Microorganisms were isolated from the body cavities of four aquarium fish belonging to several species (*Corydoras aeneus*, *Xiphophorus hellerii*, *Platypoecilus maculatus*, *Lebistes reticulatus*) all of which had died suddenly within a period of a week. One strain (1) was obtained from a living, infected *Corydoras aeneus*.

We are indebted to Dr. E. K. Borman, Bureau of Laboratories, Connecticut State Department of Health, Hartford, for preliminary antigenic typing and for his advice and comments, and to Dr. S. H. Hutner, Haskins Laboratories, New York, and to Dr. R. W. Hinshaw, Camp Detrick, Md., for advice and reading of the manuscript.

DESCRIPTION

In all cases observed, lesions developed on one side of the body as small areas of greenish discoloration just under the skin between the pectoral and ventral fins. Upon incision, a fetid and purulent material was exuded. A Gram stain of the discharge revealed numerous Gram-negative rods, 1.3 to 2 microns long and 0.7 micron wide, with rounded ends, and exhibiting bipolar staining.

In broth and on agar, the bacteria were arranged singly, in pairs, and occasionally in filaments. The rods were encapsulated, as determined by Anthony's method, and did not form endospores. Active motility was observed and single polar flagella were demonstrated by Novel's method (1939). The organisms were facultatively anaerobic but grew best with unrestricted access to air. In nutrient broth, the optimum growth temperature was 37° C., the maximum 43° C., and the minimum 5° C. The *p*H range for growth was from 5.0 to 9.5 with the optimum range between 6.5 and 7.5; best growth occurred at *p*H 7. Cultures maintained for almost 8 months in the refrigerator still contained viable organisms.

On nutrient agar, colonies averaged 2 mm. in diameter in 24 hours. They were circular, smooth, entire, slightly convex, and opaque. On blood agar, strains 1 and 2 exhibited a beta hemolytic zone averaging 7 mm. in diameter in 24 hours. After 48 hours the colonies were surrounded by a 16-mm. hemolytic zone with a greenish-brown

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discoloration. Strain 3 was nonhemolytic. Blood-agar and nutrient-agar cultures had a strong odor similar to that detected in the incised lesion from the infected fish. Colonies on eosin-methylene-blue and MacConkey's agars appeared glistening and colorless. On agar slants, growth was abundant, filiform, glistening, butyrous, and colorless. The appearance of the medium remained unchanged. In nutrient broth a pellicle was formed; there was dense clouding and a scant flocculent sediment. A loopful of a 48-hour broth culture inoculated into nutrient broth resulted in visible growth in 4 hours at 37° C.

At 20° C., growth in gelatin was best at the top, with subsequent stratiform liquefaction. At 37° C., liquefaction was complete in 24 hours. Nitrates were rapidly reduced to nitrites. The methyl-red test was negative. Acetylmethylcarbinol and indole were produced by strains 1 and 2. Strain 3 produced acetylmethylcarbinol but failed to form indole. Growth occurred on Simmon's citrate agar. In Koser's citrate broth, strain 1 was negative, strains 2 and 3 positive, after 3 days. Hydrolysis of cornstarch was complete in 24 hours (no color with iodine). Digestion of egg albumin and Loeffler's serum slants began in 48 hours and was practically complete in 96 hours. H_2S was not produced in Pb acetate medium or in Kligler's iron agar. The urease test was negative. There was slight acid production (*p*H 6) with the formation of a small amount of precipitate in bromocresol-purple milk. Peptonization was evident in 48 hours and practically completed in 120 hours. Litmus milk was reduced in 24 hours.

Various sugars, alcohols, and glucosides were sterilized by filtration through a porcelain filter and incorporated into standard basal medium in 1-percent concentrations. On original isolation, strains 1 and 2 produced acid in lactose after 21 days, and culture 3 after 27 days. This conforms to the behavior of the paracolon type of microorganisms which are described as slow lactose fermenters in Bergey's Manual (Breed, Murray, and Hitchens, 1948). After several serial transfers in lactose broth, the time of acid formation in lactose was reduced by 6 to 11 days, depending on the strain. Readings made during a 4-week period showed that acid and gas were produced in 24 hours at 25° C. and at 37° C. in L-arabinose (weak), glucose, D-fructose, D-mannose, sucrose, maltose, trehalose, soluble starch, dextrin, glycogen, and mannitol. A faint acid reaction and trace of gas appeared in salicin after 4 to 6 days' incubation at 37° C. At 25° C., salicin was fermented by all 3 strains in 24 hours. Strain 3 differed from strains 1 and 2 in that it produced acid and gas in raffinose but not in sucrose at either temperature. No acid or gas was produced in D-xylose, L-rhamnose, cellobiose, mellibiose, melizitose, inulin, glycerol, erythritol, adonitol, dulcitol, D-sorbitol, inositol. and esculin, at 25° C. or 37° C.

Preliminary antigenic analysis indicated that strains 1 and 2 were antigenically diverse from any of the paracolon types described by Stuart et al. (1943). Strain 1 proved to possess somatic components XXX and XL of the *Salmonella* group, while all tests with flagellar antisera were negative. Strain 2 was rough and consequently could not be typed. Strain 3 was negative for somatic components I to XXXVIII and for all flagellar antigens.

PATHOGENICITY

Nine goldfish (*Carassius auratus*) and 27 adult white mice were inoculated intraperitoneally with 0.2 ml. of 24-hour broth cultures of the 3 strains, and in 19 hours all were dead. In every instance, the organisms were reisolated in almost pure culture from the fluid present in the body cavity. Similar tests were made with 0.2-ml. suspensions of heat-killed bacteria and filtrates from the same 24-hour nutrient broth cultures. All fish and mice proved refractory. Strain 3 also proved to be pathogenic for guinea pigs.

Further experiments were carried out at the Microbiological Laboratory, Kearneysville, W. Va., in which 60 fingerling trout were used. Ten trout of each of the following species were inoculated intraperitoneally with 0.2 ml. of a 24-hour broth culture: Rainbow trout (*Salmo gairdnerii*), eastern brook trout (*Salvelinus fontinalis*), and brown trout (*Salmo trutta*). As controls, 10 fish of each of these species were inoculated with sterile broth. The temperature of the water in the troughs was approximately 14° C. Some deaths

occurred within 24 hours after inoculation, and in less than 41 hours eight of the rainbow and all the brook trout were dead. All controls of these two species lived. Results of the brown-trout inoculations were not as striking as those of the other two species. In 48 hours six of the brown trout had died, but three of the control fish also were dead. There were no further deaths in either group.

Gross pathological changes were observed in inoculated yearling trout of the three species. Inoculations were performed, as previously indicated, using strain 1. Dead and living fish were examined. Macroscopically, the artificially infected trout did not show any external lesions other than slight swelling and congestion in the anal region. The most important and characteristic internal pathological changes noted were as follows: Intestine filled with a yellow or white gelatinous mucus, particularly in the posterior portion; blood vessels congested and intestinal wall swollen; liver redder in color than in the controls, and spleen enlarged and much darker. There was some exudate in the peritoneal cavity and occasionally the peritoneum in the posterior portion of the abdominal cavity was congested.

DISCUSSION

Biochemically, the organisms described in this paper are very similar to *Paracolobactrum aerogenoides;* but the possession of a single polar flagellum would place the bacteria in the genus *Pseudomonas*. It is interesting to note that cultures 1 and 2 differed from classical description of members of the *Paracolobactrum* group in that they formed indole and acetylmethylcarbinol. The production of both substances is not a common occurrence within this genus. Though the slow fermentation of lactose, its pathogenicity, and presence of some *Salmonella* somatic antigens suggests relationship to paracolons, the possession of a single polar flagellum would, according to the present taxonomic concepts, relate these organisms to the genus *Pseudomonas*.

Paracolon types have been described in warm-blooded and cold-blooded animals. Edwards, Cherry, and Bruner (1943) reported isolating a paracolon type from the liver of a rattlesnake. Hinshaw and McNeil (1946a) reported the isolation of paracolon types that caused heavy mortality in turkey poults. The same authors (1946b) isolated related paracolon types from the livers of rattlesnakes, suggesting a relationship between the types isolated from snakes and those causing infection in turkeys. Hinshaw and McNeil (1947) reported the isolation of two sucrose-fermenting paracolon types possessing antigenic components of the *Salmonella* group from Pacific fence lizards and of paracolons from gopher snakes and sick turkey poults.

Members of the genus *Pseudomonas* have been isolated repeatedly in outbreaks of disease of fishes and other cold-blooded animals (Schaperclaus 1941, Guthrie 1942). The relative frequency of isolations of these groups of bacteria from other cold-blooded animals, and the isolations described in this paper, call attention to the possibility that fish may be carriers of these microorganisms. It is also possible that the converse is true, that fish acquire infection from organisms carried by higher animals. The fact that these organisms have been shown experimentally to be pathogenic for both cold-blooded and warm-blooded animals places them in a unique position and leads one to speculate on the role played by fish with respect to infection in man.

SUMMARY

The isolation and description of a unique bacterium pathogenic for warm-blooded and cold-blooded animals is discussed.

The microorganism described in this report has a peculiar taxonomic position in that its single polar flagellum is a characteristic of the genus *Pseudomonas*, whereas relationship to the paracolons is suggested biochemically by its physical and antigenic properties. Paracolon organisms producing acetymethylcarbinol and classified as *Paracolobactrum aerogenoides* have been isolated from the gastrointestinal tract of man during epidemics, but this is believed to be the first description of an organism similar to *P. aerogenoides* pathogenic for fish.

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