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Erwinia-Like Microorganisms Isolated from Animal and Human Hosts

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ABSTRACT

MURASCHI, THELMA F. (New York State Department of Health, Albany), MILTON FRIEND, AND DOROTHY BOLLES. Appl. Microbiol. **13**:128–131. 1965.—*Erwinia*-like microorganisms were isolated from the vital organs of more than 40% of a large deer population examined as well as from 13% of a small random sampling. Over a brief period of observation, a similar isolate was recovered from throat cultures of two children and one adolescent. There is no obvious explanation of the presence of the plant pathogen or its significance under these circumstances.

The plant pathogens Erwinieae, named for Erwin F. Smith, pioneer American plant pathologist, are classed according to *Bergey's Manual* as a tribe in the family Enterobacteriaceae. They are said to invade living plants and to produce dry necrosis, galls, wilts, and soft rot. Reports of the *Erwinia* include definition of characteristics (Winslow et al., 1917; Billing and Baker, 1963) and studies for differentiation from the coliform bacteria which they so closely resemble (Stanley, 1938; Stuart, Griffin, and Baker, 1938; Stuart, Mickle, and Borman, 1940; Dowson, 1941; Elrod, 1941, 1942).

No reference to isolation of any members of the tribe of Erwinieae from animal or human source was found in the literature. This is a report of *Erwinia*-like microorganisms isolated from vital organs of a large percentage of a wild deer population studied as well as from throat cultures of three children, raising the question of the position of this presumed saprophyte in animal and human disease.

MATERIALS AND METHODS

Animal isolates. Concern of the New York State Conservation Department over an apparent reduction in the number of fawn among deer known to populate the Seneca Ordnance Depot, Seneca County, New York, prompted an investigation to determine the possible role of bacterial infection. Specimens of liver and spleen of 141 deer were collected from animals at the Seneca Ordnance Depot, made available in much the same manner as for earlier studies (Reilly, Muraschi, and Dean, 1962). Specimens were also obtained from 15 additional deer found in rural localities in Upstate New York.

Human isolates. During a brief period of observation, three isolates as suspect species of *Flavobacterium* were referred from a diagnostic service performing examinations of throat cultures for isolation of hemolytic streptococci. One patient was a 2-year-old male; the others were 6- and 15-year old females. Two were cases with clinical diagnoses of tonsillitis, the other with hypertrophied tonsils.

Blood serum. Specimens were obtained from 53 of the 141 deer in the above group.

Bacteriological procedures. Samples of approximately 8 by 8 mm of the two organs from an individual animal were washed with phosphate-buffered saline (*p*H 7.2) and ground with sterile alundum in a mortar. Macerated tissue was suspended in buffer solution for inoculation of blood-agar plates (beef infusion and cysteine). Plates, incubated at 35 C in an atmosphere containing CO_2, were examined for growth after 1, 2, and 4 days.

Reagents. Antigens of isolates used in plate tests were aqueous ether extracts prepared according to the method of Ribi, Milner, and Perrine (1959) in the isolation of the somatic antigens of *Salmonella enteritidis*; for agglutination tests, standardized formalin-killed culture suspensions were used without distinction of somatic and flagellar antigens. Antisera were produced in rabbits by the intravenous injection of formalin-treated culture suspensions. Intravenous injection of heat-killed culture induced illness in rabbits, as did injections of a concentrate of a 14-day broth culture filtrate.

Serological procedures. Isolates were examined for identification and serological relationships by gel diffusion plate tests [an adaptation of the method of Ouchterlony (1948, 1949)]. Plates were read for zones of precipitate after 24 and 48 hr at room temperature. Deer sera were tested for evidence of agglutinins to leptospires (Muraschi, 1959), *Listeria monocytogenes* somatic antigens of types 1 and 4b (*personal communication*, Mitchell Gray, Montana State College, Agricultural Ex-

periment Station, Bozeman, 1960), *Brucella abortus*, and *Pasteurella tularensis*, and three of the deer isolates.

RESULTS

From 113 of the 141 specimens, and 2 of the 15 specimens, a yellow chromogen was produced on culture. Morphologically, the microorganism was a gram-negative rod. Colonies were generally circular, flat, smooth, shiny, opaque, and entire. Growth in broth led to uniform turbidity with sediment.

In a study of seven representatives of the large group of isolates, the bacteria were found to be motile; to grow at 35 C, 20 C, and, after 1 week in a refrigerator, at approximately 5 C; and to cause acid reaction without gas in glucose, xylose, mannitol, sucrose, and maltose, but not lactose. Biochemical reactions are presented in Table 1. In limited tests, isolates possessed a low degree of pathogenicity for both mice and guinea pigs. Recovery was accomplished from the blood stream or the spleen and liver (or both) after death from intraperitoneal injection of concentrated culture suspension. A heat-stable toxin was produced which induced severe illness in mice and guinea pigs for 12 to 24 hr without lethal effect. In drug-sensitivity tests, resistance was demonstrated to nitrofurantoin, erythromycin, sulfisoxazole, novobiocin, oleandomycin, penicillin, ristocetin, methicillin, and vancomycin. The bacteria were sensitive to chloramphenicol, colistin, kanamycin, neomycin, polymyxin, tetracycline, and dihydrostreptomycin.

Two strains referred to the Communicable Disease Center, Atlanta, Ga., for identification were reported to resemble a culture in their collection called "*Erwinia milletiae*."

TABLE 1. *Biochemical reactions of Erwinia milletiae and Erwinia-like isolates*

Reaction	*Erwinia milletiae*	Deer isolates
Acid produced from		
Glucose	+	+
Sucrose	+	+
Maltose	+	+
Mannitol	+	+
Xylose	+	+
Lactose	−	−
Gelatin liquefaction	+	+
Pectate medium liquefaction*	−	−
Indole production	−	−
Catalase production	+	+
Oxidase production	−	−
Esculetin production	+	+
Nitrate reduction	+	+

* Reagent: Sunkist sodium polypectate No. 6024, Sunkist Growers, Inc., Ontario, Calif.

Bergey's Manual describes *E. milletiae* as a pathogen that causes dry necrosis and galls or wilts in plants but not a soft rot. Other species of the genus *Erwinia*, composed of microorganisms which invade tissues of living plants, are known to produce soft rots as a result of pectinases. Edwards and Ewing (1962) stated that the *Erwinia* include at least two distinct entities. The microorganisms causing galls and blights of plants fail to reduce nitrate to nitrite; the microorganisms causing soft rot of plants "generally reduce nitrate to nitrite" and "generally possess the property of liquefying sodium pectinate gel." Although the strain of *E. milletiae* available to us and the deer isolates examined reduced nitrates to nitrites, in tests for pectinases which we performed, none of the cultures exhibited liquefaction of the medium.

In the esculin test in which 7 species of *Salmonella*, 11 strains of *Escherichia coli*, and 1 strain of *Alcaligenes* sp. all failed to react (Miller and Burns, 1962), the strain of *E. milletiae* and the deer isolates exhibited production of esculetin within 18 hr.

A total of 79 isolates then available for study were examined for morphological, biochemical, and serological properties. One was nonmotile, and 10 were unlike others in biochemical reactions, mostly because of the production of acid in lactose. Serological studies indicated the presence of at least seven types into which 55 of the 68 strains could be grouped. More than half (29) of the typed strains belonged in one serological group. The next largest group consisted of eight strains; others contained two to six members (Table 2). There was evidence that some cultures were mixtures of more than one serotype. Thirteen strains were not typable; their classification would probably require production of additional antisera for recognition of common antigens. One of the isolates from a human was of the 29-deer-member type; a second was of the 5-member; and the third, a 2-member type. All isolates failed to react in tests with antiserum to the culture of *E. milletiae* received from the Communicable Disease Center. Similarly, antigen of the Communicable Disease Center strain failed to react with available antisera to deer isolates.

Agglutination tests were performed with sera available from 53 of the Seneca Ordnance Depot deer; as antigens, a deer isolate of the major serotype (that with 29 members), one of a 4-member serotype, and one of a 2-member serotype were used. Twenty-two sera showed no agglutination, 26 exhibited reaction in only low

TABLE 2. *Erwinia-like isolates from wild deer and human hosts*

Isolate source*	Strains with biochemical reactions of *Erwinia*	Serotype†							Untyped
		1	2	3	4	5	6	7	
Deer a...	66	28	4	2	8	5	4	2	13
Deer b...	2	1					1		
Human ..	3	1					1	1	

* Deer a, Seneca Ordnance Depot; deer b, five counties in New York State.

† By gel diffusion tests with aqueous ether extract antigens and antisera produced with deer isolates.

TABLE 3. *Deer sera exhibiting agglutinins to deer isolates*

Deer no.	Serum titer* with antigen			Isolate serotype
	Type 1 (isolate from deer no. 5)	Type 2 (isolate from deer no. 11)	Type 3 (isolate from deer no. 23)	
7	80	20	20	1
11	80	20	0	2
25	80	20	80	None
22	80	80	80	Untyped
23	80	80	80	Untyped
5	20	0	20	1

* Titer is expressed as reciprocal of highest serum dilution giving 2+ or stronger agglutination in test incubated overnight in a 37 C water bath.

serum dilution (1:10 to 1:20), and 5 reacted to a titer of 1:80. Relationship of agglutinin titer to isolate, where recovered, is indicated in Table 3 as well as results with serum of the animal from which the culture was isolated. No significant degree of agglutination was obtained in tests with other antigens employed.

DISCUSSION

The isolates described here resemble the bacterial genus *Erwinia* insofar as we have been able to determine, and they are, therefore, described as "*Erwinia*-like."

The significance of the recovery of *Erwinia*-like microorganisms on culture from vital organs of a minimum of 45% of a large deer population examined as well as from 13% of a random sampling is unexplained (as is the source of the microorganism in nature). On the basis of current knowledge available, the *Erwinia* are plant pathogens saprophytic for man and animals. Thus, the recovery of members of the tribe from animals without clinical disease and from human hosts with diagnoses of tonsillitis suggests only a possible role of opportunist in situations of stress to the host. Studies on animals might shed light on this subject. As an experimental animal, the rabbit would probably be well suited because in the course of antiserum production it was observed that an appreciable number of these animals exhibited evidence of sensitivity, with toxic reactions, both mild and severe.

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