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Yersinia enterocolitica and Yersinia-like Organisms Isolated from Frogs and Snails [1]

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ABSTRACT

Isolations were made of *Yersinia enterocolitica* from a leopard frog *(Rana pipiens)* and of eight other Yersinia-like organisms from two green frogs *(Rana clamitans)* and a snail *(Lymnaea palustris)*. Biochemical and serological characterizations of the isolates are presented.

INTRODUCTION

Yersinia enterocolitica is a bacterium recently designated as a distinct species.[6,8] It previously was known as: *Bacterium enterocoliticum*,[14] *Pasteurella pseudotuberculosis* type b,[5] and "Pasteurella X."[9] It is quite similar to *Pasteurella pseudotuberculosis (Yersinia pseudotuberculosis)*, but differs by rarely being pathogenic to laboratory animals,[12] not being susceptible to *P. pseudotuberculosis* bacteriophage,[11] and not fermenting rhamnose.[6] It ferments sucrose and usually decarboxylates ornithine.[6]

Since its recognition as a separate species, it has been isolated as a pathogen in mammals from a variety of geographical locations. Most of the early isolations of *Y. enterocolitica* were from dead or dying chinchillas in Europe[6,1] and North America.[16] More recently, it was also isolated from hares on the Franco-Belgian border.[13] Isolations were made from: a dog,[9] a cow,[10] a horse,[10] and pigs.[5] It has also been isolated as a pathogen from humans.[14,7-15] Based on reports of natural disease in animals, the pathology in *Y. enterocolitica* infections does not differ substantially from that in *P. pseudotuberculosis* infections.

A few isolations have been made from apparently healthy animals, indicating that these animals may serve as carriers of *Y. enterocolitica*. Strains have been isolated from the alimentary canals of pigs in France[5] and deer in Michigan.[16]

Following three isolations of *Yersinia enterocolitica* from the feces of apparently healthy deer *(Odocoileus virginianus)* in southern Michigan,[16] we attempted to isolate these organisms from some of the aquatic animals inhabiting the ponds, marshes, and swamps of this area.

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Study Area

The Edwin S. George Reserve, University of Michigan, is a 1200-acre tract of land about 25 miles northwest of Ann Arbor in Livingston County, Michigan. The vegetation of the reserve is approximately 35% woodlots (predominantly oak-hickory), 2% brushlands, 40% grassy uplands, and 23% ponds, swamps, bogs, and marshes.[2]

MATERIALS AND METHODS

Collections

Collections were made of leopard frogs (*Rana pipiens*), green frogs (*Rana clamitans*), bullfrogs (*Rana catesbeiana*), painted turtles (*Chrysemys picta*), Blanding's turtles (*Emydoidia blandingi*), and four species of snails (*Aplexa hypnorum, Lymnaea palustris, Helsisoma campanulatum,* and *Helisoma trivolvis*). The collections were made from May 7 to July 9, 1966 by netting from a boat or the shore.

Processing Collected Material

Each frog was pithed and opened ventrally. Two to three mls of fecal material were transferred aseptically from the colon to a screw-capped test tube containing Trypticase Soy Broth,[5] 0.5% Yeast Extract,[6] and 50 μg/ml potassium tellurite (TSB/YE/Tel). These specimens were then stored at 4°C for five to six months.

The turtles were decapitated and the plastron was removed with a bone saw. Fecal material was aseptically transferred to TSB/YE/Tel and stored five to six months at 4°C.

The whole snails were crushed in a mortar and pestle. The remains were also stored at 4°C in TSB/YE/Tel for five to six months.

Isolation Media

The isolations were made on Bacto-MacConkey Agar[8] and CV-3, an enriched medium developed by one of us (T.F.W.). CV-3 is composed of trypticase soy broth, 2.5% agar, 0.5% yeast extract, 20 μg/ml novobiocin, 5 μg/ml erythromycin, 200 μg/ml cycloheximide, 2.5 μg/ml crystal violet, and 5 μg/ml potassium tellurite.

Biochemical Characterization

Biochemical characterization of the microorganisms was based on the *Manual for the Identification of Medical Bacteria.*[3] The cytochromoxidase reaction was determined with Path Tec-CO test strips.[7]

Serology

All serological studies were done by hemagglutination of sensitized, nontanned sheep erythrocytes.[4] Antigens were prepared from the isolates and evaluated against antisera to *Pasteurella pseudotuberculosis* and *Yersinia enterocolitica* strains. The *P. pseudotuberculosis* antisera were made against I-B strain Alaska, I-B strain 2950, II-A strain 1328, II-B strain 769, III (Mollaret strain unidentified), IV (strain unidentified), and V strain 25. The *Y. enterocolitica* antisera were made against a pig strain (PA 120), a hare strain (Lucas 110), three George Reserve deer strains (M1195a, M1215a, and M1237a), two New York human strains (33114 and 5819), several chinchilla strains (P76, P71, A905A(2), and P370), and a human strain P106. Two strains with Wetzler's serofactor #13 (I-1 Chr. and K-168) and an *E. coli*-like strain (JDM-66) were also included.

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[6] Difco Laboratories, Detroit, Michigan.
[7] General Diagnostic Division, Warner-Chilcott Laboratories, Morris Plains, New Jersey.
[8] Difco Laboratories, Detroit, Michigan.

RESULTS

Strain 5F4-32 was isolated from a leopard frog. Strains 2F33-41, 2F33-42, 2F35-32, and 2F35-33 (2F33 and 2F35 series) were isolated from two green frogs. Strains 5M4-41, 5M4-42, 5M4-43, 5M4-44 (5M4 series) were isolated from a snail (*Lymnaea palustris*).

Biochemical Reactions

All isolates were Gram negative, cytochromoxidase negative, catalase positive, nonhemolytic, nonsporing rods. They produced an acid butt and alkaline slant, but no gas from glucose, nor H_2S on Kligler's Iron Agar[8]. They were motile at 24°C, but nonmotile at 37°C. They were urease positive, phenylalanine deaminase negative, gelatinase negative, ONPG positive, methyl red positive, and indole negative. They did not decarboxylate lysine, arginine, nor glutamic acid. They reduced nitrates to nitrites, hydrolyzed starch, and grew in KCN broth at 24°C and 37°C. The isolates varied in the acetylmethylcarbinol, malonate, citrate, alkaline phosphatase, and ornithine decarboxylase reactions (Table 1). In the ornithine decarboxylase test, the isolates of the 2F33 and 2F35 series were negative; strain 5F4-32 and the strains of the 5M4 series grew in the medium, but did not give a distinct positive or negative reaction.

All isolates were fermentative. They all acidified arabinose, xylose, glucose, mannose, maltose, trehalose, salicin, glycerol, mannitol, and sorbitol in 48 hours. All acidified cellobiose within 4 days. All isolates produced a small amount of gas. None of the isolates acidified melezitose nor glycogen in 14 days. Reactions varied among the individual strains with regard to lactose, rhamnose, melibiose, sucrose, raffinose, amygdalin, dulcitol, and erythritol (Table 1). All isolates hydrolyzed aesculin in 24 hours.

TABLE. 1 *Biochemical variation between frog and snail isolates*

Text	Isolate								
	5F4 -32	2F33 -41	2F33 -42	2F35 -32	2F35 -33	5M4 -41	5M4 -42	5M4 -43	5M4 -44
Acetylmethylcarbinol	+	—	(+)	—	—	+	—	—	+
Malonate utilization	—	+	+	+	+	—	—	—	—
Citrate utilization	+	+	+	+	+	+	+	+	—
Alkaline phosphatase	—	(+)	(+)	(+)	+	—	—	—	—
Ornithine decarboxylase	NC	—	—	—	—	NC	NC	NC	NC
Carbohydrate acidification (14 days)									
Lactose	—	+	+	+	+	+	+	+	+
Rhamnose	—	+	+	+	+	+	+	+	+
Melibiose	—	+	—	—	+	+	+	+	+
Sucrose	+	—	(+)	—	—	+	+	+	+
Raffinose	—	—	—	+	+	(+)	—	+	(+)
Amygdalin	+	—	—	—	—	+	+	+	+
Dulcitol	—	+	+	+	+	—	—	—	—
Erythritol	+	(+)	(+)	(+)	(+)	—	—	—	—

+: positive reaction; (+): weak positive reaction; —: negative; NC: growth, but no change in the indicator.

Serology

The results of the serological studies are summarized in Table 2. Strain 5F4-32 has a strong serological relationship to the New York human strain 33114, and to a lesser degree with New York human strain 5819. The isolates of the 2F33 and 2F35 series have some antigens in common with the Mexican chinchilla strain A905A(2). The 5M4 series has little serological identity with any of the *P. pseudotuberculosis* or *Y. enterocolitica* strains considered.

Pathogenicity in Mice

A limited amount of animal pathogenicity was done. Eight spartan mice were inoculated intraperitoneally with 0.2 ml of an 18 hour culture of 5F4-32. Three mice received 180,000 organisms, three received 1800 organisms, and two mice received 18 organisms. One animal receiving 180,000 microorganisms was dead on the 17th day. No attempt was made to re-isolate the organism. No other mice died in the 21 day period of observation.

Strain 2F33-41 was inoculated into eight spartan mice; three received 132,000 organisms, three received 1320 organisms, and two received 13 organisms. No deaths occurred in the 21 day period of observation.

Strain 5M4-43 was also inoculated into eight spartan mice; three received 78,000 organisms, three received 780 organisms, and two mice received 8 organisms. No deaths occurred in the 21 day observation period.

DISCUSSION

Biochemically and serologically, strain 5F4-32 is a *Yersinia enterocolitica*. The lack of a distinct positive ornithine decarboxylase reaction as well as the slight gas production is unusual; but in his study of 55 *Y. enterocolitica* strains, Frederiksen[6] found variation among the strains on these same criteria. Wetzler and Hubbert[16] have recently discussed the problem of gas production among some *Y. enterocolitica* strains.

The three isolates of *Y. enterocolitica* from deer showed two distinct biochemical and serological profiles. Since 5F4-32 was different from both of them, it may be concluded that there are at least three distinct populations of *Y. enterocolitica* on the George Reserve.

Strain 5F4-32, to our knowledge, is the first *Y. enterocolitica* from a cold-blooded vertebrate. All other reported isolations have been made from mammals.

The isolates of the 5M4 series and the 2F33 and 2F35 series resemble *P. pseudotuberculosis* and *Y. enterocolitica* sufficiently to be tentatively included in the genus *Yersinia*.

TABLE 2. *Reciprocal antibody titers of* Pasteurella pseudotuberculosis *and* Yersinia enterocolitica *antisera to frog and turtle isolates.*

Antigens of frog & snail isolates	*P. pseudotuberculosis* antisera									*Y. enterocolitica* antisera												*E. coli*-like anti serum
	I-B Alaska	I-B 2950	II-A	II-B	III	IV	V	I-1 Chr.	K-168 Chr.	PA 120	Lucas 110	M1195a	33114	P 76	P 71	5819	M1215a	A905A(2)	M1237a	P 106	P 370	JDM-66
5F4-32	0*	20	0	0	0	0	0	0	80	0	0	0	640	40	0	160	0	0	0	0	0	0
2F33-41	0	10	0	0	40	10	0	0	10	20	0	0	0	0	10	10	0	80	0	0	0	0
2F33-42	10	0	0	0	20	0	0	10	0	20	0	0	0	0	10	0	0	20	0	0	0	0
2F35-32	0	10	0	10	40	10	0	10	20	20	10	0	0	20	10	10	0	80	0	0	0	0
2F35-33	0	0	0	10	40	10	0	10	10	20	0	0	0	0	10	0	0	160	0	0	0	0
5M4-41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5M4-42	0	0	0	0	0	0	0	0	20	0	10	0	0	0	0	0	10	0	0	0	20	0
5M4-43	0	20	0	10	0	0	10	10	40	0	20	0	0	80	20	40	20	0	20	10	40	0
5M4-44	0	0	0	10	0	0	0	0	20	0	20	0	0	10	0	10	0	0	0	0	20	0

0* - negative at a 1:10 dilution of antiserum

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