Edwardsiella ictaluri sp. nov., the Causative Agent of Enteric Septicemia of Catfish

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We characterized 13 cultures of the enteric bacterium causing enteric septicemia of catfish by studying their biochemical reactions, deoxyribonucleic hybridizations, and deoxyribonucleic acid guanine-plus-cytosine contents. We confirmed that this bacterium is a new species, which is most closely related to *Edwardsiella tarda* of the family *Enterobacteriaceae*. Five strains of the bacterium causing enteric septicemia of catfish were 80% or more related to the type strain, SECFDL GA 77-52 (= CDC 1976-78 = ATCC 33202), in 60°C deoxyribonucleic acid homology reactions. Species level relatedness among the 13 strains which we studied was demonstrated by the more than 80% relatedness in 75°C reactions. The bacterium causing enteric septicemia of catfish was most closely related to *E. tarda* (56 to 62%) in 60°C reactions. The guanine-plus-cytosine was 53 mol%, as determined by buoyant density centrifugation. We propose the name *Edwardsiella ictaluri* sp. nov. for the bacterium causing enteric septicemia of catfish.

Enteric septicemia of catfish (ESC) is a newly described bacterial disease primarily of cultured channel catfish (*Ictalurus punctatus*) (9). The causative agent of this disease is a gram-negative, rod-shaped, 0.5- by 1.25- μ m, oxidase-negative, peritrichous, fermentative bacterium that has been isolated 26 different times from the kidney tissues of moribund catfish. Biochemically, this organism is most similar to *Edwardsiella tarda* but differs from *E. tarda* in several key diagnostic characteristics (1).

ESC was first detected in 1976. Although not always consistent, the clinical signs of this disease in *I. punctatus* are typical of the acute bacterial septicemias caused by *E. tarda*, *Aeromonas hydrophila*, and *Pseudomonas fluorescens* (5, 11).

ESC presently constitutes an economic threat to catfish farmers in Alabama, Georgia, and Mississippi. The pathogenicity of the ESC bacterium to species of fish other than catfish has not been demonstrated, although it has been isolated from channel catfish (*I. punctatus*), white catfish (*Ictalurus catus*), and brown bullhead (*Ictalurus nebulosus*). In this paper we characterize, classify, and name the ESC bacterium.

MATERIALS AND METHODS

Bacterial strains. Since the initial isolation of the ESC bacterium in July 1976, a total of 24 strains has

been isolated from diseased fish in Alabama and Georgia by the Southeastern Cooperative Fish Disease Laboratory, Auburn University. Two additional isolates were received by the Southeastern Cooperative Fish Disease Laboratory from Maryland and Mississippi for identification in 1978. These 26 cultures represented approximately 12% of all reports of fish diseases of bacterial etiology recorded from January 1976 to October 1979 at the Southeastern Cooperative Fish Disease Laboratory. We used 13 of these strains in this study (Table 1).

Methods. Cellular morphology was determined by Gram staining and flagellation was determined by using the modified Leifson stain (Becton, Dickenson & Co.). Motility was determined in wet mounts with

TABLE 1. Sources of the strains on which the description of the ESC bacterium is based

Centers for Disease Control no.	SECFDL ^a no.	Source	Geographic origin
1975-78	GA 78-27	Channel catfish	Georgia
1976-78	GA 77-52	Channel catfish	Georgia
1977-78	AL 78-118	Channel catfish	Alabama
2917-78	GA 78-33	Channel catfish	Georgia
2918-78	AL 78-119	Channel catfish	Alabama
2919-78	AL 78-120	Channel catfish	Alabama
2920-78	AL 78-123	Channel catfish	Alabama
2921-78	AL 78-125	Channel catfish	Alabama
2922-78	AL 78-126	Channel catfish	Alabama
2923-78	AL 78-145	Channel catfish	Alabama
2924-78	GA 78-36	Channel catfish	Georgia
2925-78	MD 78-1	White catfish	Maryland
2926-78	MS 78-1	Channel catfish	Mississippi

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phase-contrast microscopy and in semisolid glucose motility deep cultures (16). The optimum growth temperature for each isolate was determined by inoculat-

ing glucose motility deep cultures by vertical stabbing with a needle and incubating these cultures for 96 h at 4, 10, 12, 15, 20, 25, 30, 35, 37, and 40°C. The temper-

TABLE 2. *Biochemical characteristics of 13 strains of E. ictaluri sp. nov.*

Characteristic	Reaction of type strain (GA 77-52) ^a	No. of strains giving opposite reaction
Gram strain	—	0
Motility at 25°C	+	1 ^b
Motility at 37°C	—	0
Flagellation	+	0
Cytochrome oxidase	—	0
Nitrate reduction to nitrite	+	0
O/F test (25°C)	+/+ ^c	0
Pigment	—	0
<i>o</i> -Nitrophenyl- β -D-galactopyranoside	(+)	0
H ₂ S (from triple sugar iron agar)	—	0
Urease	—	0
Indole	—	0
Methyl red	(+)	4 ^d
Voges-Proskauer	—	0
Citrate (Simmons)	—	0
KCN	—	0
Gelatin (22°C)	—	0
Arginine dihydrolase	—	0
Lysine decarboxylase	+	0
Ornithine decarboxylase	+	7 ^e
Ornithine (Minitex; 48 h)	—	0
Phenylalanine deaminase	—	0
Deoxyribonuclease	—	0
D-Glucose	+	0
Gas from D-glucose at 25°C	+	0
Gas from D-glucose at 37°C	—	0
Lactose	—	0
Sucrose	—	0
D-Mannitol	—	0
Dulcitol	—	0
Salicin	—	0
Adonitol	—	0
i-Inositol	—	0
D-Sorbitol	—	0
L-Arabinose	—	0
Raffinose	—	0
L-Rhamnose	—	0
Malonate	—	0
Maltose	+	0
D-Xylose	—	0
Trehalose	—	0
Glycerol	(+)	2 ^f
Starch	—	0
Fructose	+	0
Galactose	+	0
D-Mannose	+	0
Sorbose	—	0
Erythritol	—	0
Ribose	+	0
Amygdalin	—	0
Arbutin	—	0
Esculin	—	0
Cellobiose	—	0
Inulin	—	0
Melezitose	—	0
Dextrin	—	0

^a Symbols: +, Positive reaction within 48 h; (+), positive reaction between 3 and 9 days; —, negative reaction after 7 days. The incubation time and temperature were 48 h and 37°C, respectively, unless otherwise noted.

^b Strain MS 78-1.

^c Acid produced oxidatively and fermentatively in Hugh and Leifson O/F medium.

^d Strains GA 78-36, AL 78-120, AL 78-126, and MD 78-1.

^e The following strains gave a delayed positive reaction after 2 to 3 days at 37°C: GA 78-33, AL 78-119, AL 78-125, AL 78-120, AL 78-123, AL 78-126, and MS 78-1.

^f Strains GA 77-52 and GA 78-27.

ature at which motility and acid production were most pronounced was considered to be optimum.

The antimicrobial agent susceptibility of each isolate was determined by the agar diffusion method (2), but we used sensitivity agar (Difco Laboratories) rather than Mueller-Hinton agar due to poor growth of the isolates on Mueller-Hinton agar.

Biochemical testing was done by the methods of Edwards and Ewing (6) and Hickman and Farmer (10). Additional testing was conducted by using the Analytab 20E system (Analytab Products) and the Minitek system (BBL Microbiology Systems).

Antisera for a serological comparison of the ESC strains and a stock culture of *E. tarda* obtained from the National Fish Health Center, Kearneysville, W. Va., were prepared in adult channel catfish by using heat-killed bacteria. The fish were bled at the conclusion of a 6-week injection schedule. Sera from each group were pooled and refrigerated at 4°C. Control serum was obtained from preinjection bleedings. Slide agglutination tests were performed by using two-fold dilutions of antiserum mixed with an antigen suspension calibrated to a no. 10 McFarland barium chloride standard.

The strains and techniques used to prepare and label deoxyribonucleic acids (DNAs) and to determine DNA relatedness have been described previously (3).

Determinations of guanine-plus-cytosine contents was by buoyant density centrifugation in cesium chloride (D. H. Lewis, personal communication).

RESULTS AND DISCUSSION

The ESC bacterium is a gram-negative rod-shaped organism which measures 1.75 by 5.0 µm after 6 h of culture on solid media and 0.5 by 1.25 µm after 18 to 48 h of culture (A. Scott, personal communication). This organism is peritrichous; all strains tested except strain MS 78-1 were motile at 25°C but not at 37°C; strain MS 78-1 was not motile at either temperature.

The biochemical reactions of the 13 strains of the ESC bacterium tested were almost identical (Table 2). The optimum growth temperature for the ESC bacterium was between 25 and 30°C. Growth was slow on blood agar plates; at 30°C this organism took 48 h to form typical colonies 2 mm in diameter. All strains produced gas from glucose at 25°C but not at 37°C, and all strains were negative for indole production and H₂S production from triple sugar iron agar. Six of the strains were positive for ornithine decarboxylase after 24 h, four strains were positive after 3 days, and three strains were positive after 5 days of incubation at 37°C.

The tests which differentiated the ESC bacterium from *E. tarda* were motility at 37°C, the indole test, the Jordan tartrate test, gas production from glucose at 37°C, and H₂S production from triple sugar iron agar; the ESC strains were negative for all of these characters (4, 7, 13). The ESC bacterium was differentiated from *Yersinia ruckeri* (another enteric fish pathogen) by the

delayed motility of the ESC bacterium at 25°C, by gas production from glucose at 25°C, and by negative tests for citrate utilization (Simmons), trehalose utilization, gelatin liquefaction (22°C), and tartrate utilization (Jordan) (8, 12). The tests which have diagnostic significance for the ESC bacterium are shown in Table 3.

We detected no cross-agglutination in experiments with *E. tarda* antiserum and ESC bacterium antigen or with *E. tarda* antigen and ESC bacterium antiserum. A titer of 160 was obtained with *E. tarda* antigen and the homologous antiserum, and a titer of 320 was obtained with ESC bacterium antigen and the homologous antiserum.

The guanine-plus-cytosine content of the ESC bacterium DNA was 53 mol%, as determined by buoyant density centrifugation (Lewis, personal communication).

The antimicrobial agent susceptibility of the ESC bacterium has been reported previously (9); this bacterium is susceptible to oxytetracycline, chlortetracycline, kanamycin, neomycin, nitrofurazone, and dihydrostreptomycin and resistant to erythromycin and sulfamerazine.

Table 4 shows the DNA relatedness among the ESC strains and between the ESC bacterium and other members of the family *Enterobacteriaceae*. Five strains of the ESC bacterium were 80% or more related to strain 1976-78 (GA 77-52) in 60°C reactions. There was no evidence of divergence within related sequences, as judged by the absence of decreased thermal stability within related DNA sequences. The species level relatedness among the ESC strains was evident from the more than 80% relatedness observed in the 75°C reactions, in which only very closely related DNA sequences could form duplexes.

TABLE 3. Biochemical and biophysical characteristics important in differentiating three fish pathogens belonging to the family *Enterobacteriaceae*

Characteristic	Reaction of: ^a		
	<i>E. ictaluri</i>	<i>E. tarda</i>	<i>Y. ruckeri</i>
Motility at 25°C	(+)	+	+
Motility at 37°C	—	+	—
Indole	—	+	—
Citrate (Simmons)	—	—	+
Trehalose	—	—	+
Gelatin (22°C)	—	—	+
Jordan tartrate	—	+	+
Gas from glucose at 25°C	+	+	—
Gas from glucose at 37°C	—	+	—
H ₂ S	—	+	—

^a +, Positive reaction within 48 h; (+), positive reaction between 3 and 7 days; —, negative reaction. The incubation time and temperature were 48 h and 37°C, respectively, unless otherwise noted.

TABLE 4. DNA relatedness of *E. ictaluri* strain GA 77-52 (=CDC 1976-78) to other members of the family *Enterobacteriaceae*

Source of unlabeled DNA		Relative binding ratio at 60°C ^a	% Divergence ^b	Relative binding ratio at 75°C ^c
Species	Strain			
<i>E. ictaluri</i>	1976-78	100	0.0	100
<i>E. ictaluri</i>	2929-78	97	0.0	98
<i>E. ictaluri</i>	2921-78	95	0.0	97
<i>E. ictaluri</i>	2925-78	84	0.0	84
<i>E. ictaluri</i>	1977-78	83	0.0	85
<i>E. ictaluri</i>	2918-78	81	0.0	84
<i>E. tarda</i>	5497-61	62	14.5	32
<i>E. tarda</i>	3592-64	61	12.0	28
<i>E. tarda</i>	1126-64	56	13.5	28
<i>Escherichia coli</i>	K-12	31		6
<i>Shigella boydii</i>	1610-55	28		5
<i>Salmonella typhimurium</i>	LT2	35		11
<i>Citrobacter freundii</i>	460-61	33		7
<i>Citrobacter diversus</i>	1066-71	37		8
<i>Levinea amalonatica</i>	25406	34		8
<i>Klebsiella pneumoniae</i>	2	24		11
<i>Klebsiella oxytoca</i>	13182	35		6
<i>Enterobacter aerogenes</i>	1627-66	34		9
<i>Enterobacter cloacae</i>	1347-71	33		9
<i>Enterobacter sakazakii</i>	4562-70	29		5
<i>Enterobacter gergoviae</i>	604-77	32		12
<i>Enterobacter agglomerans</i>	2780-70	34		9
<i>Enterobacter agglomerans</i>	3123-80	24		14
<i>Enterobacter agglomerans</i>	1429-71	28		12
<i>Enterobacter agglomerans</i>	1741-71	32		8
<i>Enterobacter agglomerans</i>	3482-71	28		8
<i>Enterobacter agglomerans</i>	6070-69	27		5
<i>Enterobacter agglomerans</i>	6003-71	18		9
<i>Enterobacter agglomerans</i>	5422-69	30		11
<i>Enterobacter agglomerans</i>	4388-71	41		9
<i>Enterobacter agglomerans</i>	1600-71	25		6
<i>Enterobacter agglomerans</i>	5378-71	37		10
<i>Enterobacter agglomerans</i>	219-71	40		8
<i>Enterobacter agglomerans</i>	1645-71	30		6
<i>Erwinia amylovora</i>	EA 178	30		8
<i>Erwinia nigrifluens</i>	EN 104	30		12
<i>Erwinia quercina</i>	EQ 102	24		8
<i>Erwinia rubrifaciens</i>	ER 105	17		7
<i>Erwinia salicis</i>	ES 102	25		8
<i>Erwinia carotovora</i>	495	33		12
<i>Erwinia carnegieana</i>	EC 186	25		7
<i>Erwinia chrysanthemi</i>	SR 32	15		8
<i>Erwinia cypripedii</i>	EC 155	32		9
<i>Erwinia rhapontici</i>	ER 106	30		8
<i>Erwinia mallotivora</i>	2851	30		10
<i>Serratia marcescens</i>	868-57	30		12
<i>Serratia liquefaciens</i>	446-68	31		7
<i>Serratia rubidaea</i>	934-72	31		12
<i>Serratia fonticola</i>	4556-71	33		8
<i>Serratia plymuthica</i>	392	34		13
<i>Hafnia alvei</i>	5632-72	30		7
<i>Proteus mirabilis</i>	PR 14	8		2
<i>Proteus vulgaris</i>	PR 1	6		1
<i>Proteus myxofaciens</i>	19692	7		2
<i>Providencia alcalifaciens</i>	3370-67	11		3
<i>Providencia alcalifaciens</i>	26240	10		1
<i>Providencia stuartii</i>	2896-68	8		0
<i>Providencia rettgeri</i>	1163	10		2
<i>Morganella morganii</i>	25830	24		8
<i>Yersinia enterocolitica</i>	497-70	26		2
<i>Yersinia pseudotuberculosis</i>	P 62	18		7

TABLE 4—Continued

^a Relative binding ratio = [(percentage of heterologous DNA bound to hydroxyapatite)/(percentage of homologous DNA bound to hydroxyapatite)] × 100.

^b The percent divergence was the decrease in thermal stability of heterologous DNA duplexes compared with the thermal stability of the homologous DNA duplexes. This value could be expressed as a percentage because each degree decrease in thermal stability was caused by approximately 1% unpaired bases in double-stranded DNA. In homologous controls with both labeled DNA and unlabeled DNA from *E. ictaluri*, the average levels of DNA duplex formation were 66% in 50°C reactions and 73% in 75°C reactions. These values were arbitrarily defined as 100%. Each heterologous reaction was performed twice at 60°C and twice at 75°C. Control reactions in which labeled DNA was incubated in the absence of unlabeled DNA showed an average of 5% binding of hydroxyapatite. These control values were subtracted from heterologous binding values before normalization.

The relatedness of the ESC bacterium to species of *Enterobacteriaceae* is shown in Table 4. This bacterium is most closely related to *E. tarda* (56 to 62% in 60°C reactions). In these reactions the percent divergence was 12 to 14.5%, and relatedness fell to about 30% in 75°C reactions, leaving no doubt that the ESC bacterium and *E. tarda* are separate species. The relatedness of the ESC bacterium to species of *Escherichia*, *Shigella*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Erwinia*, *Serratia*, *Hafnia*, and *Morganella* (*Proteus morganii*) was generally between 25 and 35%, and its relatedness to *Proteus* and *Providencia* species was 6 to 11%.

These DNA relatedness data and the biochemical characteristics of the ESC bacterium are consistent with placing this organism in the family *Enterobacteriaceae* as a new species. The ESC bacterium is phenotypically and genetically most closely related to *E. tarda*. Therefore, we propose the name *Edwardsiella ictaluri* sp. nov. (ic ta lu'ri. M.L. masc.n. *Ictalurus* generic name of the host catfish; M.L. gen.n. *ictaluri* of *Ictalurus*) for the ESC bacterium. The type strain of *E. ictaluri* is GA 77-52 (=CDC 1976-78 =ATCC 33202).

A comparison between *E. ictaluri* and "*Paracolobactrum anguillimortiferum*" (not on the Approved Lists of Bacterial Names [14]) was not possible due to the lack of viable strains of the latter. The data reported by Wakabayashi and Egusa (15) indicate that "*P. anguillimortiferum*" may be more closely related to *E. tarda* than to *E. ictaluri*.

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REPRINT REQUESTS

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