***Edwardsiella tarda* (*Paracolobactrum anguillimortiferum*)
Associated with Pond-Cultured Eel Disease**

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Morphological and biochemical characteristics of 22 strains of an enteric bacterium isolated from diseased pond-cultured eels in Shizuoka Prefecture in 1972 were examined.

These organisms were gram-negative, peritrichously flagellated rods. They gave negative oxidase reaction, utilized glucose fermentatively in Hugh-Leifson's medium and reduced nitrate to nitrite. Some distinguishing features of the organisms were the production of hydrogen sulfide and indol, positive to methyl red test, negative to Voges-Proskauer test, no growth on Simmons' citrate agar, growth on Christensen's citrate agar, decarboxylation of lysine and ornithine, and utilization of fructose, galactose, mannose, maltose, and glycerol (Table 1).

All of the strains examined were sensitive to dihydrostreptomycin and kanamycin, but some of them were resistant to chloramphenicol, tetracycline and colistin (Table 2).

The above properties suggest that the organism should be identified as *Edwardsiella tarda*, with which *Paracolobactrum anguillimortiferum* is thought to be synonymous.

HOSHINA¹⁾ isolated a gram-negative, peritrichously flagellated rod from diseased eels which had symptoms similar to red disease. It produced gas from glucose, but failed to ferment lactose. As the bacterium was not identical to any formerly established bacteria, he named it *Paracolobactrum anguillimortiferum* sp. nov., 1962. However, because the genus *Paracolobactrum* has been struck off from the list of genera belonging to the family Enterobacteriaceae, the necessity for reexamining the classification of the bacterium had been pointed out, but no report is yet available on this subject.

The present authors carried out a monthly investigation on epizootics of pond-cultured eels in Shizuoka Prefecture in 1972 and found that the disease caused by this bacterium was common at many ponds, particularly during the summer months, when it occasionally caused high mortalities. The purpose of this paper is to describe the morphological and biochemical characteristics of 22 strains of the bacterium which they collected during the investigation and to discuss its classification.

Materials and Methods

Materials Materials used were 22 strains which were isolated each from 22 different diseased eels from ponds at Yoshida, Shizuoka Prefecture during the period from May 11 to October 17, 1972.

Morphology Morphological and tinctorial examinations were carried out with the

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cultures grown on nutrient agar (nutrient broth 'Difco' containing 0.5% NaCl and 1.5% agar, pH 7.2) at 25°C for 18 to 48 hours. Flagella staining was conducted by Toda's method.

Cultural characteristics The tolerance ranges of NaCl were determined by using nutrient broth as a base medium. NaCl was added at various concentrations 0% to 5.0%. The dependency of the growth on pH was examined with nutrient broth into which 0.5% NaCl, 0.05 M tris (hydroxymethyl) aminoethane and 0.05 M maleic acid were added. In both the pH and the NaCl tolerance tests, growth was determined up to 5 days at 25°C.

Biochemical examinations Biochemical examinations were carried out as follows. Hydrogen sulfide production in TSI medium, SIM medium; urease test in urea broth (Eiken); indol production in tryptone water; ammonium production in nutrient broth after 5 days; methyl red and Voges-Proskauer tests in glucose phosphate peptone water after 4 days; nitrate reduction in nitrate broth after 3 days; oxidase test, KOVACS³⁾, GABBY and HADLEY³⁾; utilization of glucose, citrate and alginate as a sole carbon source in 7 days on ammonium agar (Simmons' agar base); KCN test, MÖLLER⁴⁾; gelatin liquefaction in a gelatin stab in 15 days at 20°C; hydrolysis of starch, flooding the surface of a culture on starch agar with Lugol's iodine after 4 days; decarboxylation of lysine, arginine and ornithine in 4 days, FALKOW⁵⁾; phenylalanine deaminase, EWING⁶⁾; utilization of organic acids including citrate, tartrate and mucate, KAUFFMANN and PETERSEN⁷⁾; citrate utilization on Christensen's agar; tartrate utilization on Jordan's agar; malonate utilization in Leifson's broth; lipolysis of tributylin, corn oil and triacetin, DAVIS and EWING⁸⁾; fermentation in semisolid agar base (Eiken) in 39 days for lactose, sucrose, salicin and glycerol, 2 days for others; beta-D-galactosidase test, LAPAGE and JAYARAMAN⁹⁾. These tests were carried out at both 25°C and 37°C, unless otherwise stated.

Results

Morphology The cells were gram-negative, nonsporing, peritrichously flagellated rods, measuring about $2 \times 0.6 \mu$ in smears of 18 to 48 hour old cultures on nutrient agar (Fig. 1).

Cultural characteristics Growth of the organism on nutrient agar was rather slow. Colonies after 24 hour incubation were uniformly round, about 1 mm in diameter, glistening, whitish gray, entire, convex, translucent and moist. They grew on MacConkey's agar and SS agar. In nutrient broth, they grew uniformly turbid with a little white sediment, forming no pellicle.

All the strains could grow in media with 0% to 4% NaCl and some tolerated 4.5% NaCl for growth. They could grow in media whose initial pH ranged from 5.5 to 9.0, but could not survive at either pH 4.5 or pH 9.5. Growth in nutrient broth was observed at 15°C to 42°C.

Biochemical characteristics Biochemical properties of the 22 strains are summarized in Table 1. There were no discrepancy found in biochemical properties among the 22 strains.

The sensitivity of the strains to antimicrobial agents was given in Table 2.

Fig. 1. Peritrichous flagellation of *Edwardsiella tarda* from eel.

Fig. 2. Abscessed lesion developed in the kidney of a typical 'Cho-man' diseased eel, infected with *Edwardsiella tarda*.

Discussion

The genus *Edwardsiella*, with the single species *Edwardsiella tarda*, was proposed as a new genus in the family Enterobacteriaceae by EWING *et al*¹⁰⁾. Previous to this the species had been designated as 'bacterium 1483-59'¹⁰⁾, 'Asakusa group'¹¹⁾ and 'Barthulomew group'¹²⁾.

It is our conclusion that the 22 strains isolated from pond-cultured eels should be identified as *Edwardsiella tarda*, because their morphological, cultural and biochemical properties are very good accord with that of *E. tarda*, except for inutilization of D-tartrate in Kauffmann and Petersen's broth (Table 1).

Morphological and biochemical properties of *Paracolobactrum anguillimortiferum* described by HOSHINA¹⁾ are identical to those of the present organism (Table 1), although his isolates could not be investigated in the present study, because they had been lost.

E. tarda has been isolated from reptiles¹³⁾ and warm-blooded animals¹⁴⁾ including human¹⁵⁾. The pathogenicity of the organism to animals, however, has not been established sufficiently. The pathogenicity of *P. anguillimortiferum* to the eel was first demonstrated by HOSHINA¹⁾. He showed that an injection with 0.1–0.4 mg of the cells killed eels in a few days or a week.

During the field investigation mentioned at the beginning of this report, our attention was attracted to the fact that *E. tarda* was isolated mainly from eels in which abscessed or ulcerative lesions developed in the kidney or the liver (Fig. 2). Further it was observed in a preliminary experiment that the injection of eels with *E. tarda* strains isolated from dis-

Table 1. Biochemical reactions given by 22 strains from diseased eels, compared with those of *E. tarda* and *P. anguillimortiferum*.

Test or substrate	22 strains (Eels)	<i>E. tarda</i> ¹⁰⁾	<i>P. anguillimortiferum</i> ¹⁾
Hydrogen sulfide	+	+	+
Urease	—	—	—
Indole	+	+	+
Ammonium	—	—	—
Methyl red	+	+	d
Voges-Proskauer	—	—	—
Nitrate reduction	+	+	+
Oxidase	—	—	—
Catalase	+	—	+
Ammonium agar			
Citrate	—	—	—
Glucose	—	—	—
Acetate	—	—	—
Alginate	—	—	—
KCN	—	—	—
Milk (litmus)	—	—	—
Gelatin	— (20°C)	—	—
Starch	—	—	—
Decarboxylases			
Lysine	+	+	—
Arginine	—	—	—
Ornithine	+	+	—
Phenylalanine deaminase	—	—	—
Christensen's citrate	+	+	—
Jordan's tartrate	(+)	—	—
Malonate	—	—	—
Kauffmann-Peterson broth			
Citrate	(+) (25°C) — (37°C)	+	—
D-tartrate	—	+	—
i-tartrate	—	—	—
L-tartrate	—	—	—
Mucate	—	—	—
Lipases			
Corn oil	—	—	—
Triacetin*	— (25°C)	—	—
Tributyrin	—	—	—
Hugh-Leifson's agar	F	F	—
Glucose (acid)	+	+	+
Gas from glucose	+	+	+
Arabinose (acid)	—	—	d
Rhamnose (")	—	—	—
Xylose (")	—	—	—
Fructose (")	+	—	+
Galactose (")	+	—	+
Mannose (")	+	—	+
Sorbose (")	—	—	—
Lactose (")	—	—	—
Sucrose (")	—	—	—

Table 1. (Continued)

Test or substrate	22 strains (Eels)	<i>E. tarda</i> ¹⁰⁾	<i>P. anguillimortiferum</i> ¹⁾
Maltose (")	+	+	+
Threhalose (")	—	—	—
Cellobiose (")	—	—	—
Raffinose (")	—	—	—
Starch (")	—	—	—
Dextrin (")	—	—	d
Glycogen (")	—	—	—
Inulin (")	—	—	—
Salicin (")	—	—	—
Esculin (")	—	—	—
Glycerol (")	(+)	d	—
Mannitol (")	—	—	—
Sorbitol (")	—	—	—
Dulcitol (")	—	—	—
Adonitol (")	—	—	—
Erthritol (")	—	—	—
Inositol (")	—	—	—
Beta-D-galactosidase	—	—	—

+; positive, —; negative, (+); weakly positive, d; diverse, F; fermentative.

*; Control medium also became acidic at 37°C without any contamination.

Table 2. Sensitivity to antimicrobial agents of the 22 strains of *E. tarda* (*P. anguillimortiferum*) isolated from diseased eels.
(Sensitivity Disks 'EIKEN')

Antimicrobial agents	Sensitive			Resistant
	+++	++	+	—
Penicillin	0	0	5	17
Erythromycin	0	0	0	22
Oleandomycin	0	0	0	22
Leucomycin	0	0	0	22
Chloramphenicol	21	0	0	1
Tetracycline	16	0	0	6
Dihydrostreptomycin	22	0	0	0
Kanamycin	22	0	0	0
Colistin	10	0	0	12
Sulfisoxazole	0	0	0	22

eased eels produced abscessed lesions in the above-mentioned organs but that of motile aeromonads never produced similar lesions. Therefore the disease caused by *E. tarda* can be distinguished from red disease from both etiological and pathological view points, though HOSHINA¹⁾ stated that *P. anguillimortiferum* was a pathogen of red disease. Detailed studies on the epizootiology and the pathology of the *E. tarda* infection of the eel will be reported elsewhere.

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