

## NOTES

### First Report of a Human Isolate of *Erwinia persicinus*

CAROLINE M. O'HARA,<sup>1\*</sup> ARNOLD G. STEIGERWALT,<sup>2</sup> BERTHA C. HILL,<sup>1</sup>  
J. MICHAEL MILLER,<sup>1</sup> AND DON J. BRENNER<sup>2</sup>

*Nosocomial Pathogens Laboratory Branch, Hospital Infections Program,<sup>1</sup> and  
Meningitis and Special Pathogens Branch, Division of Bacterial  
and Mycotic Diseases,<sup>2</sup> Center for Disease Control  
and Prevention, Atlanta, Georgia 30333*

Received 11 July 1997/Returned for modification 20 August 1997/Accepted 19 September 1997

*Erwinia persicinus* was first described in 1990 after being isolated from a variety of fruits and vegetables, including bananas, cucumbers, and tomatoes. In 1994, it was shown to be the causative agent of necrosis of bean pods. We now report the first human isolate of *E. persicinus*. The strain was isolated from the urine of an 88-year-old woman who presented with a urinary tract infection. By the hydroxyapatite method, DNA from this strain was shown to be 94.5% related at 60°C and 86% related at 75°C to the type strain of *E. persicinus*. The biochemical profile of *E. persicinus* is most similar to those of *Erwinia rhapontici*, *Pantoea agglomerans*, and *Enterobacter* species. It is negative in tests for lysine, arginine, ornithine, dulcitol, and urea. It is motile and positive in tests for D-sorbitol and sucrose. It is susceptible to the expanded-spectrum cephalosporins, aminoglycosides, and fluoroquinolones, but it is resistant to ampicillin, ticarcillin, and cefazolin.

*Erwinia persicinus* is a plant pathogen that was described by Hao et al. (6) in 1990. These first five strains were isolated from tomatoes ( $n = 3$ ), cucumber ( $n = 1$ ), and banana ( $n = 1$ ). In 1994, Brenner et al. (3) reported that *E. persicinus* was a senior subjective synonym for "*Erwinia nalandii*," an organism that was pathogenic for bean pods and seeds. There are no reports in the literature of human or animal isolates of *E. persicinus*.

A computer-based program at the Centers for Disease Control and Prevention aids in the identification of isolates that are submitted from state public health laboratories. When the conventional biochemical reactions for a given isolate are entered into the program, the program searches the database and returns a listing of the 50 most closely related isolates that it contains. When the reactions from this organism were entered,

three of the first six strains on the list were the hybridized *E. persicinus* strains of Hao et al. (6); two were strains, identified in this paper, which had previously been reported as "unidentified."

In this paper, we report the first human isolate of *E. persicinus* and an additional food isolate.

**Bacterial strains.** The strains used in this study are listed in Table 1. All were maintained at  $-70^{\circ}\text{C}$  in defibrinated sheep blood. All strains were passed twice on Trypticase soy agar with 5% sheep blood (Becton Dickinson Microbiology Systems, Sparks, Md.) before being used.

**Media and biochemicals.** The biochemical tests were performed on conventional media by the methods of Ewing (4) and by using some of the modifications described by Farmer et

TABLE 1. DNA relatedness of *E. persicinus* strains examined in this study

Strain	Source	Sender	% Relatedness of strain 9108-82 <sup>T</sup>		
			At 60°C	D <sup>a</sup>	At 75°C
<i>Erwinia persicinus</i> CDC 9108-82 <sup>T</sup> (ATCC 35998 <sup>T</sup> , HK 204 <sup>T</sup> )	Tomato	K. Komagata, Japan	100	0.0	100
<i>E. persicinus</i> CDC 9107-82 (HK 203)	Tomato	K. Komagata, Japan	100	1.5	98
<i>E. persicinus</i> CDC 9109-82 (HK 205)	Tomato	K. Komagata, Japan	97	1	91
<i>E. persicinus</i> CDC 9106-82 (HK 201)	Cucumber	K. Komagata, Japan	88	6.0	79
<i>E. persicinus</i> CDC 9105-82 (HK 200)	Banana	K. Komagata, Japan	81	5.0	76
<i>E. persicinus</i> CDC 4073-83	Urine	Texas State Health Department	95	3.5	86
<i>E. persicinus</i> CDC 839-82	Tuna fish	F. Untermann, Germany	93	3.5	87
<i>E. rhapontici</i> ATCC 29283 <sup>T</sup>			62	10	
<i>E. rhapontici</i> ER 106			50	10	
<i>P. agglomerans</i> 5422-69			49	10.5	

<sup>a</sup> D, divergence, calculated to the nearest 0.5%.

\* Corresponding author. Mailing address: Centers for Disease Control, Mailstop C16, Atlanta GA 30333. Phone: (404) 639-2316. Fax: (404) 639-3241. E-mail: cmo1@cdc.gov.

al. (5) and by Hickman and Farmer (7). Incubations were at 35°C, and test results were read at 24 h, 48 h, and 7 days, unless otherwise noted. Commercially available media were used whenever possible.

**Antimicrobial susceptibility.** Antimicrobial susceptibility profiles were determined for seven strains by the Kirby-Bauer disk diffusion method (8). MICs were determined by using a broth microdilution method and cation-supplemented Mueller-Hinton broth (9).

**DNA methods.** The preparation, isolation, and purification of labeled and unlabeled DNA, the method used for DNA reassociation, and the method used to separate single-stranded and double-stranded DNAs on hydroxyapatite have been described elsewhere (1, 2). The DNAs were labeled enzymatically in vitro with [<sup>32</sup>P]dCTP by using a nick-translation reagent kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) as directed by the manufacturer.

**Results and discussion.** Labeled DNA from *E. persicinus* 9108-82<sup>T</sup> was shown to be 81 to 100% related (average, 87%) to unlabeled DNA from four other confirmed *E. persicinus* strains tested in 60°C reactions (Table 1). Divergence within the related sequences averaged 3%, and the degree of relatedness in reactions at 75°C was 76 to 98% (average, 86%). *E. persicinus* was 62% related to the type strain of *Erwinia rhapontici*, 50% related to a second *E. rhapontici* strain, and 49% related to *Pantoea agglomerans*. The percent divergence to these three strains was 10.0 to 10.5. The degree of relatedness of labeled DNA from *E. persicinus* 9108-82 to that of the human strain (strain 4073-83) was 95% at 60°C, with 3.5% divergence, and 86% at 75°C. The type strain showed a similar level of relatedness to the strain isolated from tuna (strain 839-82). These relatedness values leave no doubt that the human and tuna strains are *E. persicinus*.

The biochemical profiles of the two newly identified strains are characteristic of the profiles found for *E. persicinus* strains (Table 2). Reactions that differ from those of the type strain include the methyl red, Voges-Proskauer, Simmons citrate, rhamnase, esculin, melibiose, and galactose reactions. Partial differentiation from other *Enterobacter* species, *E. rhapontici*, and *Pantoea* species is presented in Table 3. Accurate identification of *E. persicinus* in the clinical laboratory may not be possible without the use of the extended set of conventional biochemicals listed in Table 2. Hao et al. (6) reported that all five strains in their study produced a water-soluble pink pigment when grown on peptone yeast agar supplemented with 1% glucose at 20, 25, and 30°C. Of the seven strains that we studied, strains 9108-82<sup>T</sup> and 4073-83 produced pigment at 25°C and strain 9109-82 produced pigment at both 25 and 30°C on this same medium.

Strain 4073-83 was isolated from the urine of an 88-year-old female who presented with a urinary tract infection. The clinical history of the patient was extremely sparse; however, she had a history of atherosclerotic coronary vascular disease with congestive heart failure, hypertension, and diabetes mellitus. Her diagnoses also included a lower leg hematoma and stasis dermatitis. Her medications included indomethacin (Indocin; 25 mg three times daily [t.i.d.]), methyldopa (Aldomet; 250 mg t.i.d.), chlorpropamide (Diabinese; 250 mg daily), dipyrindamole (Persantine; 25 mg t.i.d.), digoxin (Lanoxin; 0.25 mg every 3rd day), hydrochlorothiazide-triamterene (Dyazide; twice daily), furosemide (Lasix; 40 mg t.i.d.), phenytoin (Dilantin; t.i.d.), and potassium chloride (Slow K; 600 mg t.i.d.) but included no antimicrobial agents. She had been receiving most of these medications for the previous 18 months. Data regarding the colony count associated with this isolate was unavailable, making its significance in this patient unclear.

TABLE 2. Conventional biochemical reactions for seven strains of *E. persicinus*

Test <sup>a</sup>	Cumulative % positive at the following times:			Reaction of ATCC 35998 <sup>Tb</sup>
	24 h	48 h	7 days	
Indole production		43		—
Methyl red		57		+
Voges-Proskauer		57		—
Citrate, Simmons		29	57	+ <sup>7</sup>
H <sub>2</sub> S production (triple sugar iron)	0	0	0	—
Urea, Christensen	0	0	0	—
Phenylalanine deaminase	0	0	0	—
Lysine, Moeller	0	0	0	—
Arginine, Moeller	0	0	0	—
Ornithine, Moeller	0	0	0	—
Motility	100	100	100	+
Gelatin hydrolysis (22°C)	0	0	0	—
Growth in KCN	0	0	0	—
Malonate utilization	14	100	100	+ <sup>2</sup>
D-Glucose				
Acid production	100	100	100	+
Gas production	0	0	0	—
Acid production from:				
D-Adonitol	0	0	0	—
L-Arabinose	57	86	100	+ <sup>2</sup>
D-Arabitol	0	0	0	—
Cellobiose	100	100	100	+
Dulcitol	0	0	0	—
Erythritol	0	0	0	—
D-Galactose	29	43	71	—
Glycerol	0	0	0	—
<i>i</i> ( <i>myo</i> )-Inositol	0	14	29	+ <sup>7</sup>
Lactose	14	14	14	—
Maltose	0	0	0	—
D-Mannitol	100	100	100	+
D-Mannose	100	100	100	+
Melibiose	43	43	43	—
α-Methyl-D-glucoside	0	0	0	—
Raffinose	71	71	71	+
L-Rhamnose	0	43	71	+ <sup>3</sup>
Salicin	100	100	100	+
D-Sorbitol	100	100	100	+
Sucrose	100	100	100	+
Trehalose	86	100	100	+
D-Xylose	0	0	0	—
Esculin hydrolysis	14	71	100	+ <sup>7</sup>
Mucate, acid production	0	0	0	—
Tartrate, Jordan	0	0	100	+ <sup>7</sup>
Acetate utilization	0	0	14	—
Lipase (corn oil)	0	0	0	—
DNase (25°C)	0	0	0	—
NO <sub>3</sub> <sup>-</sup> → NO <sub>2</sub> <sup>-</sup>	100			+
Oxidase	0			—
ONPG <sup>c</sup>	100	100	100	+
Hydrogen sulfide (PIA <sup>d</sup> )	0	0	0	—
Tyrosine clearing	0	0	0	—

<sup>a</sup> Incubation was at 35°C unless otherwise specified.

<sup>b</sup> Symbols: —, negative; +, positive. The superscript number indicate the days on which the reaction became positive.

<sup>c</sup> ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

<sup>d</sup> PIA, peptone iron agar.

Strain 839-82 was isolated from raw tuna in Berlin, Germany. The isolate produced histamine, but it is unknown if this strain was associated with a foodborne disease.

Antimicrobial susceptibility data are shown in Table 4. All seven strains are susceptible to all 23 agents tested with the exception of ampicillin, ticarcillin, and cefazolin. The suscep-

TABLE 3. Differentiation of *E. persicinus* and *Enterobacter* species

Species	% Reactions positive in tests for the following <sup>a</sup> :											
	Lysine, Moeller	Urea	Motility	Methyl red	Lactose	Sucrose	D-Sorbitol	Raffinose	Melibiose	$\alpha$ -Methyl-D-glucoside	Dulcitol	Esculin
<i>Erwinia persicinus</i>	0	0	99	57	14	100	100	71	43	0	0	71
<i>Erwinia rhapontici</i>	0	0	0	0	100	100	0	100	100	0	0	100
<i>Enterobacter aerogenes</i>	93	0	80	18	96	98	98	98	96	92	13	97
<i>Enterobacter amnigenus</i> biogroup 1	0	0	93	7	79	100	7	100	100	57	0	91
<i>Enterobacter amnigenus</i> biogroup 2	0	0	100	65	35	0	100	0	100	100	0	100
<i>Enterobacter asburiae</i>	0	72	1	100	75	100	99	71	5	95	0	92
<i>Enterobacter cancerogenus</i>	0	2	99	4	10	2	1	0	1	2	0	88
<i>Enterobacter cloacae</i>	0	73	93	18	68	97	94	90	84	85	14	47
<i>Enterobacter dissolvens</i>	0	100	0	0	0	100	100	100	100	100	0	100
<i>Enterobacter gergoviae</i>	91	93	91	6	53	99	3	95	94	1	0	98
<i>Enterobacter hormaechei</i>	0	86	55	9	21	100	3	0	0	83	86	0
<i>Enterobacter intermedium</i>	0	0	90	100	100	70	100	100	100	100	90	100
<i>Enterobacter nimipressuralis</i>	0	0	0	100	0	0	100	0	100	100	1	100
<i>Enterobacter sakazakii</i>	0	1	95	3	99	100	1	99	100	96	8	100
<i>Pantoea agglomerans</i>	0	10	84	48	39	80	27	30	32	7	11	63
<i>Pantoea ananas</i>	0	0	100	0	100	100	80	100	100	0	0	20
<i>Pantoea dispersa</i>	0	0	100	83	8	92	8	0	0	0	0	8

<sup>a</sup> Results were obtained as positive reactions on conventional media incubated at 35°C for 48 h.

TABLE 4. Antimicrobial susceptibilities of seven strains of *E. persicinus* by broth microdilution

Antimicrobial agent	MIC ( $\mu$ g/ml) <sup>a</sup>	
	50%	90%
Ampicillin	$\geq 64$	$\geq 64$
Piperacillin	32	$\geq 32$
Ticarcillin	$\geq 128$	$\geq 128$
Amoxicillin-clavulanic acid	4	4
Cefazolin	$\geq 32$	$\geq 32$
Cefotaxime	1	1
Cefotetan	1	1
Cefpodoxime	2	4
Ceftriaxone	1	4
Ceftazidime	2	2
Cefuroxime	8	16
Imipenem	1	1
Aztreonam	1	2
Gentamicin	$\leq 0.25$	$\leq 0.25$
Tobramycin	$\leq 0.25$	$\leq 0.25$
Amikacin	1	1
Ciprofloxacin	$\leq 0.06$	$\leq 0.06$
Ofloxacin	$\leq 0.25$	$\leq 0.25$
Chloramphenicol	2	4
Nalidixic acid	1	4
Sulfisoxazole	$> 32$	$> 32$
Tetracycline	$\leq 0.5$	1
Trimethoprim-sulfamethoxazole	$\leq 0.12/2.4$	$\leq 0.12/2.4$

<sup>a</sup> 50% and 90%, MICs at which 50 and 90% of strains are inhibited, respectively.

tibilities for these last three antimicrobial agents are at the threshold of the intermediate-resistance breakpoint.

Data for *E. persicinus* are not included in the database of any commercially available identification product. If inoculated into any of these products, the isolate would most likely be identified as *P. agglomerans*. Such isolates should be examined further to determine whether they are *E. persicinus* so that the full spectrum of disease associated with this species can be further defined.

## REFERENCES

- Brenner, D. J., P. A. D. Grimont, A. G. Steigerwalt, G. R. Fanning, E. Ageron, and C. F. Riddle. 1993. Classification of citrobacteria by DNA hybridization: designation of *Citrobacter farmeri* sp. nov., *Citrobacter youngae* sp. nov., *Citrobacter braakii* sp. nov., *Citrobacter werkmanii* sp. nov., *Citrobacter sedlakii* sp. nov., and three unnamed *Citrobacter* genospecies. Int. J. Syst. Bacteriol. 43:645-658.
- Brenner, D. J., A. C. McWhorter, J. K. Leete-Knutson, and A. G. Steigerwalt. 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. J. Clin. Microbiol. 15:1133-1140.
- Brenner, D. J., J. R. Neto, A. G. Steigerwalt, and C. F. Robbs. 1994. "*Erwinia nulanii*" is a subjective synonym of *Erwinia persicinus*. Int. J. Syst. Bacteriol. 44:282-284.
- Ewing, W. H. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier, New York, N.Y.
- Farmer, J. J., III, M. A. Asbury, F. W. Hickman, D. J. Brenner, and the *Enterobacteriaceae* Study Group. 1980. *Enterobacter sakazakii*: a new species of "*Enterobacteriaceae*" isolated from clinical specimens. Int. J. Syst. Bacteriol. 30:569-584.
- Hao, M. V., D. J. Brenner, A. G. Steigerwalt, Y. Kosako, and K. Komagata. 1990. *Erwinia persicinus*, a new species isolated from plants. Int. J. Syst. Bacteriol. 40:379-383.
- Hickman, F. W., and J. J. Farmer. 1978. *Salmonella typhi*: identification, antibiograms, serology, and bacteriophage typing. Am. J. Med. Technol. 44:1149-1159.
- National Committee for Clinical Laboratory Standards. 1997. Performance standards for antimicrobial disc susceptibility tests. Approved standard M2-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- National Committee for Clinical Laboratory Standards. 1997. Standard methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.