JOURNAL OF CLINICAL MICROBIOLOGY, July 1981, p. 79-88
0095-1137/81/070079-10$02.00/0

Vol. 14, No. 1

Tatumella ptyseos gen. nov., sp. nov., a Member of the Family *Enterobacteriaceae* Found in Clinical Specimens

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Received 30 January 1981/Accepted 24 March 1981

The name *Tatumella ptyseos* gen. nov., sp. nov., is proposed for a group of organisms (previously called group EF-9) isolated from clinical sources in the United States, Canada, and Puerto Rico. A total of 68% of these isolates were from sputum specimens. *T. ptyseos* strains are gram-negative, oxidase-negative, fermentative rods that grow on MacConkey agar. The distinctive biochemical characteristics of 44 *T. ptyseos* isolates were as follows: acid but no gas from D-glucose, sucrose, and, usually (71%), D-xylose (62% delayed); no acid from lactose, maltose, or D-mannitol; negative tests for indole, urea, methyl red, gelatin, L-lysine decarboxylase, and L-ornithine decarboxylase; L-arginine dihydrolase variable; phenylalanine deaminase positive; Voges-Proskauer positive by the Coblentz method but negative by the O'Meara method; nonmotile at 36°C but 66% weakly motile (30% delayed) at 25°C; Simmons citrate positive at 25°C (89%) but Simmons citrate negative at 36°C. Deoxyribonucleic acid-deoxyribonucleic acid relatedness studies on 26 *T. ptyseos* strains showed that they were 80 to 100% related at 60°C, which indicated that they comprise a single species. The deoxyribonucleic acid relatedness to other species within the *Enterobacteriaceae* was 7 to 38%. This is evidence that this species belongs in this family, is distinct from all described species, and is best placed in a new genus. The *T. ptyseos* isolates studied were susceptible to all of the antimicrobial agents tested by broth dilution; these antimicrobial agents were amikacin, ampicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, tetracycline, and tobramycin. Three striking differences between *T. ptyseos* and other members of the *Enterobacteriaceae* were its large zone of inhibition around penicillin (mean diameter 24 mm), its tendency to die on some laboratory media (such as blood agar) within 7 days, and its small number (usually one) of flagella. Strain H36 (=ATCC 33301, =CDC D6168, =CDC 9591-78) is the type strain of this new species. *T. ptyseos* is the type species for the genus *Tatumella*.

During the past 14 years the Special Bacteriology Section and the Enteric Section, Bacteriology Division, Centers for Disease Control (CDC), have received for identification 56 cultures of gram-negative, oxidase-negative, fermentative, rod-shaped organisms which appeared to comprise an unclassified group; the Special Bacteriology Section designated these organisms EF-9 (EF for eugonic fermenter). These strains were isolated from human clinical specimens in the United States, Canada, and Puerto Rico. The most frequent source of isolation was sputum.

These two sections performed a study to determine the possible relationship of this group to described genera and, in particular, to the family *Enterobacteriaceae*. The biochemical characteristics, antimicrobial agent susceptibilities, and deoxyribonucleic acid (DNA)-DNA relatedness of the EF-9 group were investigated. On the basis of these data, we determined that 44 of the strains represent a new species. We propose the name *Tatumella ptyseos* gen. nov., sp. nov., for these organisms.

MATERIALS AND METHODS

Bacterial strains. This study began with 56 strains, which were coded H1 through H56 for convenience; 11 of these strains were eliminated from *T. ptyseos* on the basis of biochemical characteristics, and 1 was excluded on the basis of DNA hybridization results. The remaining 44 strains were studied in detail; these strains and their sources are listed in Table 1. The one strain that differed on the basis of DNA hybridization results was H34 (CDC D4607) and was

TABLE 1. *List of T. ptyseos strains*

Code no.	Clinical source	Sender	Enteric Section no.	Special Bacteriology Section no.	ATCC no.[a]
H1	Sputum	New York	9556-78	A4185	
H2	Sputum	Washington	9557-78	A7398	
H3	Sputum	Connecticut	9558-78	A7744	
H4	Throat	Michigan	9559-78	A8442	33302
H5	Throat	Pennsylvania	9560-78	A9987	
H6	Throat	Maryland	9561-78	B1360(1)	
H7	Sputum	North Carolina	9562-78	B1994	
H8	Sputum	Connecticut	9563-78	B3569	
H9	Sputum	Puerto Rico	9564-78	B5357	
H10	Sputum	Puerto Rico	9565-78	B5361	
H11	Sputum	North Carolina	9566-78	B6259	
H12	Sputum	New York	9567-78	B6312	
H13	Sputum	California	9568-78	B8915	
H14	Blood	District of Columbia	9569-78	C99	
H16	Sputum	Maryland	9571-78	C521	
H17	Sputum	Maryland	9572-78	C1678	
H18	Sputum	Washington	9573-78	C1732	
H21	Sputum	North Carolina	9576-78	C3917	
H23	Sputum	New York	9578-78	C5420	
H24	Pharynx	North Carolina	9579-78	C7121	
H25	Throat	Maryland	9580-78	C7679	
H27	Feeding tube	Michigan	9582-78	C8149	
H28	Sputum	Canada	9583-78	D2431(1)	
H29	Sputum	Maryland	9584-78	D2575	
H30	Throat	Maryland	9585-78	D2940	
H31	Blood	Maryland	9586-78	D3004	
H32	Sputum	North Carolina	9587-78	D3877	
H35	Sputum	Missouri	9590-78	D5924	
H36	Sputum	Maryland	9591-78	D6168	33301
H37	Sputum	Washington	9592-78	D6441	
H38	Sputum	Louisiana	9593-78	D6463	
H40	Throat	Indiana	9595-78	D7051	
H41	Sputum	New York	9596-78	D7425	
H43	Sputum	Colorado	9598-78	D8819	
H44	Blood	Georgia	9599-78	D9069	
H45	Sputum	South Dakota	9600-78	E1316	
H47	Sputum	Tennessee	9172-79		
H48	Tracheal aspirate	California	9173-79		
H49	Sputum	Missouri	9174-79		
H50	Sputum	Missouri	9175-79		
H53	Sputum	Maryland	9176-79		
H54	Stool	Wisconsin	9177-79		
H55	Urine	New Jersey	9004-79	E4207	
H56	Sputum	Tennessee	9178-79	E5105	

[a] ATCC, American Type Culture Collection.

isolated from a tongue "flap." The data given in the tables are for the 44 strains of *T. ptyseos*. Culture H36 was designated the type strain because it was typical and was isolated from sputum. In this study all strains were maintained by suspending 18- to 24-h cultures in 10% skim milk, freezing these cultures at −70°C in 95% ethyl alcohol, and storing them at −70°C. All incubations were at 36 ± 1°C unless otherwise noted. Overnight cultures refer to the cultures incubated for 18 to 24 h. A total of 65 strains representing all of the relatedness groups and all known species in the *Enterobacteriaceae* were used for DNA hybridization (5, 6; unpublished data). The type strain of *Chromobacterium violaceum* (ATCC 12472) was also tested. The *Escherichia coli* strain derived from ATCC 25922 and the *Streptococcus faecalis* strain derived from ATCC 29212 were obtained from C. Thornsberry, CDC, and were used for quality control of antimicrobial agent susceptiblity tests.

Nomenclature. We used the names and classifications in *Bergey's Manual of Determinative Bacteriology*, 8th ed. (8), except that *Morganella morganii* was used rather than *Proteus morganii* and *Providencia rettgeri*, *Providencia alcalifaciens*, and *Providencia stuartii* were used rather than *Proteus rettgeri*, *Proteus inconstans* A, and *P. inconstans* B, respectively (5, 12).

Media. Whenever possible, dehydrated media from

commercial sources were used. The media used for biochemical tests done in the Enteric Section (10, 12, 13) and in the Special Bacteriology Section (14, 17) at the CDC have been described previously. The formulas for three media that are not commercially available and are used in the Special Bacteriology Section are given below. Esculin agar contains the following: esculin, 1 g; ferric citrate, 0.5 g; commercial heart infusion agar (HIA), 40 g; and glass-distilled water, 1,000 ml. This medium is boiled, adjusted to pH 7.0, dispensed into tubes, autoclaved at 121°C for 15 min, and slanted. Tryptone glucose yeast extract agar contains the following: tryptone, 5 g; D-glucose, 1 g; yeast extract, 5 g; K_2HPO_4, 1 g; agar, 15 g; and distilled water, 1,000 ml. This medium is prepared in the same way as esculin agar (see above). Starch agar is HIA containing 1% soluble starch.

Morphology. Colonial morphology and hemolysis of blood were determined on HIA containing 5% defibrinated rabbit blood and Trypticase soy agar containing 5% defibrinated sheep blood. Plates were incubated for 18 to 24 h. Gram stains were performed with 18- to 24-h HIA cultures of all strains by the Hucker modified procedure (17). Flagellar morphology was determined for seven strains (H4, H12, H13, H24, H36, H38, and H55) by the Clark modification of the method of Leifson (9) and by the simplified silver-plating method of West et al. (18). For flagellum staining these strains were grown both on tryptone glucose yeast extract agar slants and in flagellum broth overnight at 25°C. Distilled, filtered water (0.2-μm nitrocellulose filter) was used for washing and suspending the cells.

Biochemical tests. The biochemical tests performed on the 44 *T. ptyseos* strains are listed in Table 2. Some tests were done at different temperatures and by different methods. The Voges-Proskauer (VP) test was done at 36 and 25°C by the following three methods: (i) the O'Meara method, (ii) the Coblentz method, and (iii) a combination of these two methods. The same medium is used for the O'Meara and Coblentz methods and is available commercially (methyl red VP medium). The differences in the two tests are in the amounts of inoculum used, the length of incubation, and the reagents added. In the O'Meara method (10) 1 ml of medium is inoculated lightly (small amount from a needle) and allowed to incubate for 2 days. After this, 1 ml of VP reagent B (0.3% creatine in 40% potassium hydroxide) is added, and the mixture is shaken well. A positive reaction is indicated by the development of an eosin pink to red color within 4 h. In the Coblentz method (17), 2 ml of medium is inoculated heavily (the broth medium is drawn into a Pasteur pipette and used to wash off the growth from an 18- to 24-h HIA slant) and then allowed to incubate for 6 h. After this, 0.6 ml of VP reagent A (5% alpha-naphthol in 95% alcohol) and 0.2 ml of VP reagent B are added. This mixture is shaken well. A postive reaction is indicated by a red color, which usually develops within 5 to 10 min. The third method for the VP test followed the O'Meara method exactly, except that both VP reagent A (0.6 ml) and VP reagent B (0.2 ml) are added, as in the Coblentz method. We refer to this method as the modified O'Meara method. The following tests were also performed at both 25 and 36°C: methyl red, motility, Simmons citrate, L-lysine decarboxylase, L-arginine dihydrolase, L-ornithine decarboxylase, and acid from cellobiose, melibiose, D-xylose, and raffinose. Tests for acid production from carbohydrates and related compounds were performed in fermentation broth base to which Andrade indicator was added (10). Dextrin, D-fructose, D-galactose, glycogen, inulin, melezitose, and soluble starch were filter sterilized as previously described (12) and then added to the sterile basal medium at final concentrations of 1%, except for D-galactose, which was added at a final concentration of 0.5%. Other carbohydrates and their concentrations have been described previously (12). Esculin hydrolysis was determined in esculin broth (10) and on esculin agar slants. Nitrate reduction was detected in a semisolid agar medium at 24 h (10) and in heart infusion broth (HIB) containing 0.2% KNO_3 at 48 h. The following three media were used to detect indole production at 48 h: (i) peptone water (tested with Kovacs reagent [10]); (ii) tryptone broth (tested with Erhlich-Boehme reagent after xylene extraction); and (iii) HIB (tested with Erhlich-Boehme reagent after xylene extraction [17]). Gelatin tests were performed at 22°C in nutrient gelatin (10) and at 36°C in infusion gelatin (17).

Since a large number of biochemical tests were performed on these organisms, initially these tests were done by using a multi-inoculator. This saved time because it allowed all of the cultures to be inoculated at one time (Fig. 1). This method was developed by J.J.F. The multi-inoculator consisted of a series of 72 needles which transferred inocula to a test medium. All inoculations were done in a vertical biological safety cabinet (model B6000-1; The Baker Co., Inc., Sanford, Maine). To use the inoculator, we prepared and sterilized a rack of tubes (13 by 100 mm), each containing 4 ml of a particular medium. The inoculations into this medium were made from a rack containing overnight cultures in HIB by first immersing the needles in the cultures and then transferring the adhering broth to the test medium. The rack was then covered with a polypropylene cover, placed in a sterile polypropylene bag to prevent contamination, and incubated at the appropriate temperature. The multi-inoculator was constructed at CDC in the Machine Shop. The racks were obtained from Scientific Products, McGraw Park, Ill. The covers were polypropylene trays obtained from Bel-Art Products, Pequannock, N.J. The bags were autoclavable disposable bags (approximately 30.5 by 61.0 cm) from York Scientific, Inc., Ogdensburg, N.Y. The individual tubes were not covered; therefore, the urea medium was overlaid with mineral oil to prevent ammonia from escaping into adjacent tubes. Some media could only be inoculated manually. These included potassium cyanide broth and triple sugar iron agar. Any tests that gave variable results or were difficult to read were repeated on all strains by manual inoculations. These tests were VP (all methods), Simmons citrate, phenylalanine, motility, L-lysine, L-arginine, L-ornithine, cellobiose, melibiose, raffinose, salicin, D-xylose, and esculin (broth). When one strain gave an atypical reaction for a particular test, the test was repeated manually. Tests of one nitrate-negative strain (H28) and one 10% lactose-

TABLE 2. *Biochemical reactions of 44 T. ptyseos strains and the type strain*

Test	Cumulative % of strains positive at:		Type strain H36	
	1 to 2 days	3 to 7 days	Reaction[a]	Day reaction became positive
Indole	0	ND[b]	–	
Methyl red	0	ND	–	
VP:				
O'Meara method	0	ND	–	
Coblentz method	100	ND	+	
Simmons citrate				
25°C	75	89	+	2
36°C	0	0	–	
H_2S on TSI[c]	0	0	–	
Acid butt in TSI	100	100	+	
Urea	0	0	–	
Phenylalanine	98	100	+	
L-Lysine (Møller)	0	0	–	
L-Arginine (Møller)				
25°C	5	30	–	
36°C	0	14	–	
L-Ornithine (Møller)	0	0	–	
Motility				
25°C	36	66	+	
36°C	0	2	–	
Gelatin (22°C)	0	0	–	
Gelatin (36°C)	0	0	–	
Growth in KCN	0	0	–	
Malonate	0	0	–	
D-Glucose				
Acid	100	100	+	
Gas	0	0	–	
Fermentation (OF medium)	98	100	+	
Acid from:				
Adonitol	0	0	–	
L-Arabinose	0	0	–	
D-Arabitol	0	0	–	
Cellobiose	0	7	–	
Dextrin	0	0	–	
Dulcitol	0	0	–	
Erythritol	0	0	–	
D-Galactose	59	100	+	2
Glycerol	7	98	+	4
Glycogen	0	0	–	
i-Inositol	0	0	–	
Inulin	0	0	–	
Lactose	0	0	–	
Maltose	0	0	–	
D-Mannitol	0	0	–	
D-Mannose	100	100	+	
Melezitose	0	0	–	
Melibiose	27	80	+	3
α-CH_3-glucoside	0	0	–	
Raffinose	11	11	–	
L-Rhamnose	0	0	–	
Salicin	55	98	+	4
D-Sorbitol	0	0	–	
Starch	0	0	–	
Sucrose	98	100	+	
Trehalose	93	100	+	
D-Xylose	9	71	+	
Esculin hydrolysis				
Broth	0	86	+	4
Agar	98	98	+	
Mucate	0	0	–	
Jordan tartrate	0	0	–	
Acetate	0	0	–	
Lipase (corn oil)	0	0	–	
Deoxyribonuclease				
25°C	0	0	–	
36°C	0	0	–	
$NO_3^- \rightarrow NO_2^-$	98	ND	+	
Oxidase	0	ND	–	
ONPG[d]	0	0	–	
Catalase	100	ND	+	
Pectate	0	0	–	
Citrate (Christensen)	50	100	+	4
H_2S in PIA[e]	0	0	–	
Tyrosine clearing	0	0	–	
Growth at:				
25°C	100	100	+	
36°C	100	100	+	
42°C	0	0	–	
10% Lactose	2	2	–	
Starch hydrolysis	ND	0	–	

[a] –, Negative at end of the appropriate incubation period or 7 days; +, positive at 24 h or at the time of the test.
[b] ND, Not determined.
[c] TSI, Triple sugar iron agar.
[d] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.
[e] PIA, Peptone iron agar.

positive strain (H29) were repeated.

Tyrosine agar, esculin agar, deoxyribonuclease agar, 10% lactose agar, tryptone glucose yeast extract agar, starch agar, and blood agar (HIA plus rabbit blood) for the oxidase test were inoculated with the multisyringe applicator described below. Growth at different temperatures was determined on tryptone glucose yeast extract agar, and the amount of growth was recorded as light, moderate, or heavy (14). The oxidase test was performed directly on blood agar plates by the standard method and by the Kovacs method (17), using a 0.5% aqueous solution of tetramethyl-*p*-phenylenediamine dihydrochloride. The test for starch hydrolysis on starch agar was performed at 7 days by flooding the plate with Gram iodine (17).

Growth on plating media. The tests for growth on plating media listed in Table 3 were performed by using 3-ml syringes filled with overnight HIB cultures of the strains. All 44 strains could be inoculated simultaneously onto a single plate (20 by 150 mm) containing 60 ml of the medium to be tested; 1 drop (approximately 0.01 ml) of each of the 44 strains was applied simultaneously by using the multisyringe applicator previously described for bacteriophage typing

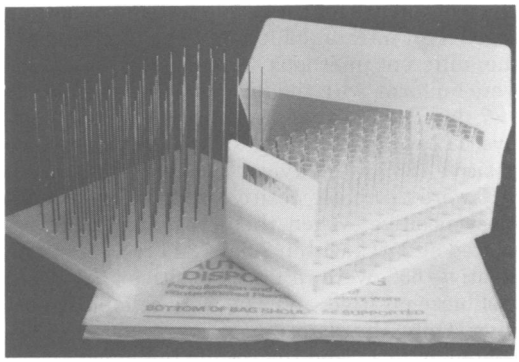

FIG. 1. *Multi-inoculator used for biochemical tests.*

TABLE 3. *Growth and appearance of T. ptyseos strains on plating media used in clinical laboratories*

Agar[a]	% Positive after 2 days	Appearance of colonies
MacConkey	100	Colorless
Brilliant green	96	Green
TCBS	0	
Hektoen	0	
XLD	5	Yellow
Tergitol 7	100	Red
EMB	100	Purple or metallic green sheen or both

[a] TCBS, Thiosulfate citrate bile salt sucrose; XLD, xylose lysine deoxycholate; EMB, eosin methylene blue.

of *Salmonella typhi* (11). Plating efficiencies were not determined on these media. All results were recorded as light, moderate, or heavy, indicating the size and amount of growth. The color and appearance of the growth were also noted.

Antimicrobial agent susceptibility testing. Antibiograms were performed on Mueller-Hinton agar by the disk method of Bauer et al. (1). The following antimicrobial agent disks were used: amikacin, 10 μg; ampicillin, 10 μg; carbenicillin, 100 μg; cephalothin, 30 μg; chloramphenicol, 30 μg; colistin, 10 μg; gentamicin, 10 μg; kanamycin, 30 μg; nalidixic acid, 30 μg; penicillin, 10 U; streptomycin, 10 μg; sulfadiazine, 0.25 mg; tetracycline, 30 μg; tobramycin, 10 μg; and trimethoprim plus sulfamethoxazole, 1.25 plus 23.75 μg, respectively. *E. coli* derived from ATCC 25922 was used for quality control. All zone sizes were measured in millimeters.

Determinations of the minimal inhibitory concentrations of antimicrobial agents were performed in Sensititre AP02 plates for gram-negative organisms (GIBCO Diagnostics, Lawrence, Mass.). The antimicrobial agents and ranges tested were as follows: amikacin, 0.25 to 32 μg/ml; ampicillin, 0.25 to 32 μg/ml; carbenicillin, 4 to 512 μg/ml; cephalothin, 1 to 128 μg/ml; chloramphenicol, 0.5 to 64 μg/ml; gentamicin, 0.12 to 16 μg/ml; kanamycin, 0.5 to 64 μg/ml; tetracycline, 0.25 to 32 μg/ml; and tobramycin, 0.12 to 16 μg/ml. Since *T. ptyseos* strains grow poorly in Mueller-Hinton broth, we used HIB for the minimal inhibitory concentration determinations. An overnight culture on Trypticase soy agar containing 5% sheep blood was suspended in HIB to a turbidity of 0.5 McFarland standard and then diluted in HIB (1:1,000) to obtain approximately 10^5 viable cells per ml. A 50-μl amount of this dilution was inoculated into each well by using a GIBCO E-1003 multichannel inoculator and E-1004 tips, which inoculated eight wells in one step. The Sensititre plates were read after 24 h according to the instructions of the manufacturer. *E. coli* derived from ATCC 25922 and *S. faecalis* derived from ATCC 29212 were used as controls. Both controls were tested in Mueller-Hinton broth and HIB; the results obtained were within the ranges of the minimal inhibitory concentrations for these organisms that are recommended by the manufacturer of the Sensititre plates.

DNA hybridization. DNA hybridizations were performed and assayed on hydroxyapatite by previously described methods (2–7). The relatedness of a test strain to the reference strain H36 was expressed as a percent hybridization value. Strains with a hybridization value of 70% (or more) at 60°C or of 50% (or more) at 75°C are usually considered as belonging to the same species. Radioactively labeled DNA from *T. ptyseos* type strain H36 was prepared and tested to determine its relatedness to the unlabeled DNAs of 26 selected strains in the EF-9 group, including strain H34, 65 strains of the *Enterobacteriaceae*, and *C. violaceum*. The 25 *T. ptyseos* strains that were chosen included 18 strains that phenotypically differed slightly from the "wild-type" strain, strain H36, and from seven other wild-type strains. *C. violaceum* was included because biochemically it closely resembles *T. ptyseos*. Unlabeled DNA from the *T. ptyseos* strains was prepared specifically for this study; however, stock unlabeled DNA preparations were used for the other strains. All DNA preparations were stored over chloroform at 4°C.

Guanine plus cytosine content of DNA. The percentage of guanine plus cytosine in DNA was determined by buoyant density (cesium chloride) centrifugation (16) in the laboratory of Manley Mandel, M.D. Anderson Hospital, Houston, Tex. Unsheared DNA was extracted at the CDC from strains H4 and H36 and sent to M. Mandel for guanine plus cytosine determinations.

RESULTS

Sources of strains. Of the 44 cultures of *T. ptyseos*, 30 (68%) were isolated from sputum, 6 (14%) were from throat cultures, 3 (7%) were from blood, and 1 each was from a tracheal aspirate, a feeding tube, a pharynx, a stool, and urine (Table 1). These strains were referred to our laboratories from 18 states in the United States, the District of Columbia, Puerto Rico, and Canada.

Morphology. At 24 h the colonies of *T. ptyseos* strains on HIA containing 5% rabbit blood were 0.5 to 1.0 mm in diameter, low convex with

entire edges, semitranslucent, smooth, and glossy. There was no change in the rabbit cells after 24 h; however, a "greening" was observed on sheep blood. The strains grew well at 25 and 36°C but did not grow at 42°C. Strain H29 had a yellow insoluble pigment within 3 days at 36°C. On HIA *T. ptyseos* cells were gram-negative, short to medium, straight rods with occasional filaments (Fig. 2). These cells were approximately 0.6 to 0.8 by 0.9 to 3.0 μm (dried and Gram stained). A total of 36% of the strains were weakly motile at 25°C within 1 to 2 days, and an additional 30% became motile by 7 days (Table 2). Only a few motile cells could be detected in wet preparations of the 24-h cultures used for flagellum staining (Fig. 2), and flagella were difficult to demonstrate. The flagellum stains revealed single polar, single subpolar, and single lateral flagella (15). Rarely, we observed cells with either two polar flagella or with a polar flagellum and a lateral flagellum. We also found a very rare cell with three flagella (one polar, one subpolar, and one lateral).

Biochemical reactions. Table 2 shows the results of the biochemical tests, along with the results for the type strain. Only one value is given in Table 2 for the indole test and one value is given for nitrate reduction, since the results of the different methods agreed. The reactions were uniform, with the following few exceptions: at 7 days 14% of the strains were arginine positive, 11% were raffinose positive, 71% were xylose positive (84% were xylose positive at 21 days), 86% were melibiose positive, and 7% were cellobiose positive. When these reactions were repeated at 25°C, within 7 days 30% were arginine positive, 65% were raffinose positive , 98% were melibiose positive, and 37% were cellobiose positive. Only the xylose-negative tests were repeated at 25°C, and the results remained xylose negative. One of the xylose-negative strains, strain H4, was deposited in the American Type Culture Collection (ATCC 33302). Strain H45 was salicin negative and esculin negative at both 36 and 25°C. Strain H28 was nitrate negative by both methods at 36°C but was nitrate positive in HIB at 25°C. All of the *T. ptyseos* strains were phenylalanine positive; however, this reaction was not as strong as that observed with *Proteus* and *Providencia*. The phenylalanine test for strain H40 was performed at 4 days because this strain grew more slowly than the other strains. None of the strains were Simmons citrate positive at 36°C, but 89% were positive at 25°C in 7 days. Esculin hydrolysis was more rapid on esculin agar slants than in broth. Strain H29 was the only yellow-pigmented strain and was the only strain that gave a weak acid reaction on 10% lactose (14). However, it did not produce acid in lactose broth and was *o*-nitrophenyl-β-D-galactopyranoside negative. All of the strains were VP positive by the Coblentz method, but when the O'Meara method (10) was used, all strains were VP negative. When the modified O'Meara method was used 100% of the strains were VP positive. The difference in the results obtained with the Coblentz and O'Meara methods was due to the addition of alpha-naphthol.

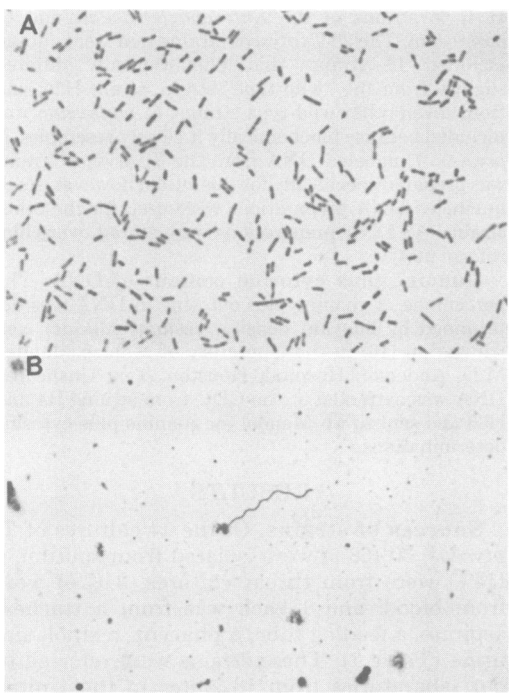

FIG. 2. *(A) Gram stain of T. ptyseos strain H36 grown on HIA for 18 to 24 h. ×3,724. (B) Silver flagellum stain of strain H4 grown on tryptone glucose yeast extract agar at 25°C for 18 to 24 h, showing a single subpolar flagellum. ×3,724.*

Growth on plating media. Growth on the differential and selective plating media used in enteric bacteriology is shown in Table 3. Within 2 days all of the strains grew on MacConkey, Tergitol 7, eosin methylene blue, and brilliant green agars; however, none grew on thiosulfate citrate bile salt sucrose or Hektoen agar, and only 5% grew on xylose lysine deoxycholate agar. On eosin methylene blue 55% of the strains had purple colonies, and 45% had metallic green colonies after 1 day. Within 2 days, five of the strains with purple colonies developed a metallic green sheen. Only two strains (H1 and H2) grew on xylose lysine deoxycholate agar, and they grew poorly.

Antimicrobial agent susceptibility tests. The results of the antibiotic tests done by the disk method are shown in Table 4. Five (11%) of the strains had no zone of inhibition around sulfadiazine, and one of these strains (H18) also had no zone around trimethoprim plus sulfamethoxazole. Otherwise, the strains were inhibited to some degree by all of the antimicrobial agents tested, including penicillin. The results obtained with the disk method appeared to be in good agreement with the minimal inhibitory concentration results (Table 5). However, the zones obtained with *T. ptyseos* were larger than the zones usually obtained with other members of the *Enterobacteriaceae* and may have resulted from the poor growth of this organism on Mueller-Hinton agar. Thus, the agreement between the disk diffusion and minimal inhibitory concentration tests may not be as good as it first seemed. The strains were sensitive to all of the antimicrobial agents tested by the broth dilution method. The minimal inhibitory concentrations of colistin, nalidixic acid, penicillin, streptomycin, sulfadiazine, and trimethoprim plus sulfamethoxazole were not determined.

TABLE 4. *Susceptibility of 44 T. ptyseos strains as determined by agar diffusion*

Antimicrobial agent	Zone diam (mm)		
	Range	Mean	Standard deviation
Amikacin	16–28	22	3.3
Ampicillin	24–41	32	4.6
Carbenicillin	22–44	32	5.0
Cephalothin	26–46	34	4.6
Chloramphenicol	28–46	33	4.2
Colistin	18–23	19	1.7
Gentamicin	19–33	26	3.8
Kanamycin	18–36	27	4.1
Nalidixic acid	23–35	28	3.0
Penicillin	15–36	24	4.6
Streptomycin	18–32	23	3.2
Sulfadiazine	6–28	17	5.3
Tetracycline	15–26	22	2.7
Tobramycin	17–26	24	4.6
Trimethoprim + sulfamethoxazole	6–30	22	5.0

TABLE 5. *Minimal inhibitory concentrations of nine antimicrobial agents*[a]

Antimicrobial agent	Cumulative % of strains inhibited at the following concn (μg/ml):					
	0.12	0.25	0.5	1	2	4
Amikacin		59[b]	93	100		
Ampicillin		86	100			
Carbenicillin						100
Cephalothin				98	100	
Chloramphenicol			32	95	100	
Gentamicin	67	95	100			
Kanamycin			100			
Tetracycline		9	30	98	100	
Tobramycin	59	93	100			

[a] All 44 *T. ptyseos* strains were tested.

[b] The first value for each antimicrobial agent is for the lowest concentration tested.

DNA hybridization. Tables 6 and 7 show the DNA relatedness data. The results obtained with the 25 *T. ptyseos* strains are shown in Table 6. These strains were 80 to 100% related to strain H36 at 60°C and 68 to 100% related at 75°C. Six strains (H11, H13, H24, H32, H47, and H55) were 68 to 78% related to strain H36 at 75°C and showed a percent divergence of 5.4 to 7.8%. Both the decrease in relatedness at 75°C and the 5 to 8% divergence indicated moderate divergence between these six strains and strain H36. The other 19 *T. ptyseos* strains were 89 to 100% related to H36 at 75°C, with percent divergences of 0 to 0.5%, indicating almost total relatedness and essentially no divergence within related se-

TABLE 6. *DNA relatedness of strain H36 to 25 other T. ptyseos strains*

Source of unlabeled DNA	% Hybridization at 60°C	% D[a]	% Hybridization at 75°C
H36	100		100
H3	100	0.2	96
H4	98	0.0	97
H5	97	0.2	96
H7	94	0.1	95
H8	99	0.0	93
H11	80	5.5	70
H13	88	5.4	70
H16	92	0.2	90
H18	96	0.3	94
H21	97	0.2	95
H24	80	7.8	69
H28	95	0.1	100
H29	89	0.1	89
H31	100	0.0	100
H32	85	6.7	68
H37	93	0.1	95
H43	92	0.2	92
H44	96	0.2	94
H45	99	0.4	99
H47	88	6.8	73
H48	97	0.3	92
H49	100	0.5	94
H50	99	0.1	94
H55	85	6.8	73
H56	95	0.5	87

[a] %D, Percentage of divergence. This value was calculated by assuming that each 1°C decrease in thermal stability of a DNA duplex was caused by 1% unpaired bases within that duplex (2). For example, consider organisms A and B that are 50% related. An A-A duplex has a mean thermal stability of 90°C, and an A-B duplex has a mean thermal stability of 80°C. The divergence value in related DNA is 10%.

TABLE 7. *DNA relatedness of type strain H36 to other T. ptyseos strains and other organisms*

Source of unlabeled DNA	% Relatedness to labeled DNA of strain H36 at:	
	60°C	75°C
T. ptyseos (25 strains)	80-100[a]	68-100
C. violaceum	2[b]	ND[c]
Citrobacter	28-30	ND
Edwardsiella tarda	25	ND
Enterobacter	24-38	ND
Erwinia and *Pectobacterium*	25-34	ND
Escherichia	25-30	ND
Hafnia	22-32	ND
Klebsiella	27-28	ND
Morganella morganii	26	ND
Proteus and *Providencia*	7-12	ND
Salmonella typhimurium	29	ND
Serratia	22-27	ND
Shigella boydii	24	ND
Yersinia	22-28	ND

[a] Range of arithmetic means.
[b] Arithmetic mean. A single value indicates that only one strain was tested.
[c] ND, Not determined.

quences. Therefore, on the basis of percent relatedness, there appeared to be two groups of strains. However, both groups were placed in the same species, since their relatedness values at both 60°C (70%) and at 75°C (60%) were more than the minimum values observed in strains of a given species (3, 5). Strain H11, H13, H32, H47, and H55 were raffinose positive at 36°C, and strain H24 was xylose negative; otherwise, these strains were phenotypically similar to strain H36. The yellow-pigmented strain H29 was 89% related to strain H36 at 60 and 75°C. Strain H34 (not included in Table 1) was 66% related to strain H36 at 60°C and 44% related at 75°C. This strain was not placed in the species *T. ptyseos*, but may belong to a second species within the genus *Tatumella*. Biochemically, this strain resembled *T. ptyseos* strains, except that it was nitrate negative and phenylalanine negative.

Table 7 shows the DNA relatedness data of strain H36 to other genera. Strain H36 was 22 to 38% related to strains of *Enterobacteriaceae*, with the exception of *Proteus* and *Providencia*, to which it was 7 to 12% related. *Proteus* and *Providencia* are known to be distantly related to other members of the *Enterobacteriaceae* (5). Of the 65 organisms tested, strain H36 was most closely related (38%) to one of the *Enterobacter agglomerans* DNA hybridization groups. Only 2% relatedness was found between *T. ptyseos* and *C. violaceum*. All 65 strains are not listed in Table 7; to save space, strains are grouped according to described genera. This table is intended to give an overall picture of the relatedness of *T. ptyseos* to other members of the *Enterobacteriaceae*. *T. ptyseos* was 27% related to *E. coli*, the type species of the type genus of this family.

Guanine plus cytosine content of DNA. The guanine plus cytosine ratios for strains H4 and H36 were 53 and 54 mol%, respectively. This is well within the range for the *Enterobacteriaceae* (5).

DISCUSSION

Biochemical and DNA relatedness studies indicated that 44 of the 56 strains originally designated EF-9 represent a single species, which belongs in the family *Enterobacteriaceae* and is distinct from any described genus or species in the family. We propose *Tatumella ptyseos* as a new genus and species within the *Enterobacteriaceae* for this group of organisms. The generic name is derived from the name of Harvey Tatum, a former co-worker and an outstanding microbiologist who worked in both the Enteric and Special Bacteriology Sections of CDC before retiring. *Tatumella* (pronunciation, Tay tum ell' uh) is a neo (modern) Latin feminine noun formed by adding the diminutive ending "-ella" to the noun "Tatum." Since the most frequent source of isolation of this organism has been sputum, we propose the specific epithet *ptyseos* (pronunciation, tie' see us); this epithet is treated as a modern Latin genitive noun derived from the Greek noun *ptyseos*, meaning "a spitting." Strain H36 (=ATCC 33301, =CDC D6168, =CDC 9591-78) is the type strain for this species. *T. ptyseos* is the type species for the genus *Tatumella*. We propose the following citations of the genus and species, which recognize the greater contributions of three of the authors: *Tatumella* Hollis, Hickman and Fanning; *Tatumella ptyseos* Hollis, Hickman and Fanning.

A description of the genus *Tatumella* follows. The proposed genus has only one species; therefore, the description is a combined generic and specific description. The cells are gram-negative rods which are 0.6 to 0.8 by 0.9 to 3.0 μm. Some filaments may be formed. Endospores have not been observed. One strain, strain H25, is motile at 36°C (3 to 5 days), and 66% are weakly motile (3 to 7 days) at 25°C. Flagella are difficult to demonstrate, but they appear to be single polar, subpolar, and lateral and, rarely, bipolar.

On blood agar at 24 h, colonies are 0.5 to 1.0 mm in diameter, low convex with entire edges, semitranslucent, smooth, and glossy. The colonies are not as large as those of most species of the *Enterobacteriaceae*, but *T. ptyseos* strains do grow on MacConkey, eosin methylene blue,

Tergitol 7, and brilliant green agars. Growth occurs at 25 and 36°C, but not at 42°C.

T. ptyseos strains ferment D-glucose, produce catalase, reduce nitrate to nitrite, and are positive for phenylalanine and VP (Coblentz method). Oxidase, urease, indole, L-lysine decarboxylase, L-ornithine decarboxylase, and gelatin tests are negative. The L-arginine dihydrolase reaction is variable. Acid is produced from D-glucose, sucrose, trehalose, salicin, D-mannose and (usually) D-xylose, melibiose, and glycerol without gas formation. No acid is produced from D-mannitol, lactose, or maltose. Simmons citrate tests are usually positive at 25 but not 36°C. The guanine plus cytosine content of the *T. ptyseos* type strain is 54 mol%.

T. ptyseos is susceptible as determined by the broth dilution method to amikacin, ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, tetracycline, and tobramycin. Because *T. ptyseos* grows poorly on Mueller-Hinton agar, categorizing it as sensitive, intermediate, or resistant by the disk diffusion method in the same way that rapidly growing *Enterobacteriaceae* are categorized may not be appropriate.

The most frequent source of isolates has been sputum. Strains have also been isolated from the throat and pharynx, from blood, from a tracheal aspirate, from a stool, from urine, and from a feeding tube. One strain which was isolated from sputum and was referred to our laboratory was reported to have been in nearly pure culture, and three strains from sputum and one strain from a throat were isolated with other flora present.

Table 8 shows some characteristics useful for presumptive identification of *T. ptyseos*. For a detailed description of the genus, see Tables 2 through 5.

Our description of *T. ptyseos* is largely compatible with the definition of the *Enterobacteriaceae*. The main difference is flagellation. Other motile members of the *Enterobacteriaceae* have peritrichous flagella. Peritrichous flagella have been defined as flagella which are arranged haphazardly around the whole cell (15). The motility of *T. ptyseos* is weak, and flagella are difficult to demonstrate. Usually, only one flagellum, (either polar, subpolar, or lateral) is observed per cell. Only rarely are cells with two or three flagella observed. This organism can be identified without performing a flagellum stain. Another distinct characteristic of *T. ptyseos* is that it grows more lightly and does not survive on laboratory media as well as other members of the *Enterobacteriaceae*. At the beginning of this study, we found that two of nine strains received in the Enteric Section had become nonviable in our stock media (10, 13). These two strains were 3 and 5 years old. All of the 37 Special Bacteriology Section strains had been frozen at −40°C in defibrinated rabbit blood for up to 14 years and were viable. Although a controlled study was not done, we found that *T. ptyseos* did not survive on blood plates for more than 1 week. We recommend that this organism be maintained frozen at −70 to −40°C. The large zone of inhibition around penicillin (average size diameter, 24 mm) is also an unusual characteristic and may result because *T. ptyseos* grows poorly on Mueller-Hinton medium. Most of the members of the *Enterobacteriaceae* that have been tested in the Enteric Section are either resistant to penicillin or have smaller zones of inhibition (unpublished data); however, *S. typhi* is known to have a fairly large zone of inhibition with penicillin (12).

The biochemical reactions of *T. ptyseos* are most similar to those of *E. agglomerans* and *C. violaceum*. This organism can be differentiated from *E. agglomerans* on the basis of its mannitol-negative reaction. The type strain of *T. ptyseos* was 38% related to one DNA hybridization group of *E. agglomerans*. *T. ptyseos* can be distinguished from *C. violaceum* because *T. ptyseos* is VP positive, gelatin negative, and often xylose positive. *T. ptyseos* DNA was essentially unrelated to DNA from the type strain of *C. violaceum*.

TABLE 8. *Characteristics useful for presumptive identification of T. ptyseos*

Characteristic	% Positive[a]
Gram-negative rod, no spores	100
Oxidase	0
Growth on MacConkey agar	100
Acid butt in TSI[b]	100
D-Glucose	
Acid	100
Gas	0
Acid from:	
D-Xylose	9 (62)
D-Mannitol	0
Lactose	0
Sucrose	100
Urea	0
Indole	0
VP (Coblentz method)	100
Phenylalanine	98 (2)
L-Lysine decarboxylase	0
L-Arginine dihydrolase	0 (14)
L-Ornithine decarboxylase	0
Gelatin	0

[a] Percent positive in 1 to 2 days. Parentheses indicate percentages delayed for 3 to 7 days.

[b] TSI, Triple sugar iron agar.

We have a limited amount of clinical information on 14 of the patients from whom *T. ptyseos* was isolated. One of the three blood isolates was from an 8-year-old male with leukemia, and one was from a male (age unknown) with an abdominal tumor; however, there was no information on the third blood isolate. The tracheal aspirate was from a 15-year-old male who developed pneumonitis after heart surgery. Other clinical diagnoses and associated illnesses included asthmatic bronchitis, pharyngitis (source, pharynx), ataxia telangiectasis, neuromuscular disease (source, feeding tube), Wegener granulomatosis, pneumonia, pulmonary edema, chronic lung disease, seizures (etiology unknown), respiratory failure, and gastroenteritis (source, stool). Of the 30 patients whose sex is known, 19 were male, and 11 were female. Of the 20 patients whose age is known, 14 were adults and 6 were children (15 years old or younger). The clinical importance of this organism is not known. However, the small amount of clinical information available suggests that it may be an opportunistic pathogen, since the immune systems of several of the patients were compromised. The incidence of *T. ptyseos* is unknown. Perhaps this report will stimulate others to search for this organism.

ACKNOWLEDGMENTS

We thank Thomas MacAdoo and Lillian Holdeman, Virginia Polytechnic Institute, Blacksburg, for their help in selecting the specific epithet and J. G. Holt, Iowa State University, Ames, for his assistance with the genus name. We also thank Arnold Steigerwalt, CDC, for extracting the *T. ptyseos* DNA and Manley Mandel, M.D. Anderson Hospital, Houston, Tex., for determining the guanine plus cytosine contents. We express our appreciation to Carolyn Baker, Jana Swenson, and Clyde Thornsberry, Antimicrobics Investigation Section, CDC, for their helpful advice on performing and interpreting the antibiotic susceptibility tests.

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