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Providencia rustigianii: a New Species in the Family *Enterobacteriaceae* Formerly Known as *Providencia alcalifaciens* Biogroup 3

F. W. HICKMAN-BRENNER,[1]* J. J. FARMER III,[1] A. G. STEIGERWALT,[2] AND DON J. BRENNER[2]

Enteric Bacteriology Section[1] and Molecular Biology Laboratory,[2] Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

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The name *Providencia rustigianii* sp. nov. is proposed for a group of organisms previously known as *Providencia alcalifaciens* biogroup 3. By DNA hybridization, strains of *P. rustigianii* were 81 to 99% related to each other at 60°C, but only 44 to 49% related to *P. alcalifaciens* biogroups 1 and 2 and 26 to 33% related to *Providencia stuartii*. *P. rustigianii* could be differentiated from *P. alcalifaciens* and *P. stuartii* by simple biochemical tests. *P. rustigianii* produced acid from D-galactose but not from trehalose; *P. stuartii* produced acid from both; and *P. alcalifaciens* produced acid from neither. *P. rustigianii* could be distinguished from *Providencia rettgeri* (formerly *Proteus rettgeri*) by urea hydrolysis and acid production from D-arabitol; *P. rustigianii* was negative for these two tests, but *P. rettgeri* was positive. Strains of *P. rustiganii* were 32 to 34% related to strains of *P. rettgeri*. Three of the 11 strains of *P. rustigianii* were isolated from stools, but the sources of the other isolates are unknown. Three strains (27%) were sensitive to colistin, and 82 to 100% were sensitive to ampicillin, carbenicillin, cephalothin, gentamicin, kanamycin, nalidixic acid, streptomycin, and tetracycline. Strain ATCC 33673 (CDC no. 0132-68) is the type strain for this species.

In 1972, Ewing et al. (4) studied 891 strains of *Providencia* and divided them into two species and six biogroups (Table 1) based on gas production from D-glucose and acid production from adonitol and *i*-inositol. This classification was used for several years; however, in 1978, DNA hybridization experiments (2) indicated that revisions would be required. Strains of *Providencia alcalifaciens* biogroups 1 and 2 were found to be highly related to each other (74 to 100% at 60°C) but only 44 to 49% related to strains of biogroup 3. In addition, strains of biogroup 4 were indistinguishable from *Providencia stuartii* (75 to 89% related by DNA hybridization at the stringent temperature of 75°C). Brenner et al. thus proposed that biogroup 4 of *P. alcalifaciens* be reclassified as *P. stuartii*. Although biogroup 3 was considered a different species (2), a formal name was not proposed because simple tests were not available to differentiate it from biogroup 4. In the same paper, *Proteus rettgeri* was reclassified as *Providencia rettgeri*. The purpose of the present study was to find simple biochemical tests that correlated with the DNA relatedness data. In this paper, we propose the name *Providencia rustigianii* sp. nov. for the group of strains formerly known as *P. alcalifaciens* biogroup 3 and show how simple tests can be used to differentiate the four species in *Providencia* (Table 2).

MATERIALS AND METHODS

Bacterial strains. The 11 strains of *P. rustigianii* studied are listed in Table 3. No clinical information was available on most of the strains. All were maintained on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) at room temperature (18 to 28°C). All incubations were at 36 ± 1°C unless otherwise noted.

Media and biochemical testing. Commercial media were used whenever possible. The biochemical tests (Table 4) were done by the methods of Edwards and Ewing (3), with some modifications described elsewhere (5, 6).

Antibiotic susceptibility tests. Antibiotic susceptibility was determined on all strains by agar diffusion according to the disk method of Bauer et al. (1). Four strains, 9168-79, 9169-81, 9172-81, and 9174-81, grew poorly on Mueller-Hinton agar and were tested on Mueller-Hinton agar plus 5% defibrinated sheep blood (BBL). The antibiotics used are listed in Table 5.

DNA relatedness. DNA relatedness studies had been done on eight of the *P. rustigianii* strains by Brenner et al. (2); no further genetic studies were done. The strains previously tested were 0132-68 (radioactively labeled), 9168-79, and 9170-81 through 9174-81 (see Table 3 for cross-reference of numbers used by Brenner et al.).

TABLE 1. Changes in the classification of *P. alcalifaciens* and *P. stuartii*

Biogroup[a]	Classification used in 1972[a]	Current classification	Biochemical reactions[b]				
			Gas	Adonitol	Inositol	Galactose	Trehalose
1	*P. alcalifaciens*	*P. alcalifaciens*	+	+	−	−	−
2	*P. alcalifaciens*	*P. alcalifaciens*	−	+	−	−	−
3	*P. alcalifaciens*	*P. rustigianii*	+	−	−	+	−
4	*P. alcalifaciens*	*P. stuartii*	−	−	−	+	+
5	*P. stuartii*	*P. stuartii*	−	−	+	+	+
6	*P. stuartii*	*P. stuartii*	−	+	+	+	+

[a] See reference 4.

[b] Tests for gas production from D-glucose and acid production from adonitol and *i*-(*meso*)-inositol were used in the 1972 classification; tests for acid production from D-galactose and trehalose were added for the current classification to further differentiate the species. Symbols: +, 90% or more positive; −, 10% or less positive. All reactions were at 48 h and 36°C.

TABLE 2. Differentiation of the species now recognized in the genus *Providencia*

Species	Biochemical reaction[a]					
	Urea hydrolysis	*i*-Inositol	Adonitol	D-Arabitol	Trehalose	D-Galactose
P. alcalifaciens	−	−	+	−	−	−
P. rustigianii	−	−	−	−	−	+
P. stuartii	V	+[b]	−[b]	−	+	+
P. rettgeri	+	+	+	+	−	+

[a] Symbols: +, 90% or more positive; V, 11 to 89% positive; −, 10% or less positive (all reactions at 36 ± 1°C and 48 h); the data for the carbohydrates and polyhyroxyl alcohols refer to acid production.

[b] *P. stuartii* biogroup 4 strains are inositol negative, and biogroup 6 strains are adonitol positive (Table 1), but both of these biogroups are rare.

TABLE 3. DNA relatedness of the type strain of *P. rustigianii* to other strains of *P. rustigianii* and to other *Providencia* species

Species	Strain no.			% Relatedness (60°C) to labeled DNA of *P. rustigianii* type strain[a]
	Enteric Bacteriology Section	Brenner et al.[a]	ATCC	
P. rustigianii	0132-68[b]	0132-68	33673 (type strain)	100
	9168-79	26240	29945	99
	9169-81	26250		91
	9172-81	156		90
	9173-81	1588		87
	9171-81	Hart 0:34		86
	9170-81	26260		85
	9174-81	12013	12013	81
	3574-67[b]			ND[c]
	1143-76[b]			ND
	1369-77			ND
P. alcalifaciens (4 strains)				44–49
P. stuartii (4 strains)				26–33
P. rettgeri (3 strains)				32–34

[a] See reference 2; DNA relatedness data were taken from this study.

[b] Strains 0132-68, 3574-67, and 1143-76 were isolated from human stool specimens; the sources of the other strains are unknown.

[c] ND, Not determined.

TABLE 4. Biochemical reactions of 11 *P. rustigianii* strains and the type strain

Test	Cumulative % positive at time (days)[a]:			Reaction for type strain ATCC 33673[b]
	1	2	7	
Indole		91		+
Methyl red		64		−
Voges-Proskauer		0		−
Citrate (Simmons)	0	0	0	−
H_2S on TSI	0	0	0	−
Urea	0	0	0	−
Phenylalanine	100			+
L-Lysine (Møller's)	0	0	0	−
L-Arginine (Møller's)	0	0	0	−
L-Ornithine (Møller's)	0	0	0	−
Motility	0	0	0	−
Gelatin (22°C)	0	0	0	−
Growth in KCN	91	100	100	+
Malonate	0	0	0	−
D-Glucose				
Acid	100	100	100	+
Gas	27	27	64	+
Acid from:				
Adonitol	0	0	0	−
L-Arabinose	0	0	0	−
D-Arabitol	0	0	0	−
Cellobiose	0	0	0	−
Dulcitol	0	0	0	−
Erythritol	0	0	0	−
D-Galactose	91	91	100	+
Glycerol	0	9	82	+[6]
i-Inositol	0	0	0	−
Lactose	0	0	0	−
Maltose	0	0	0	−
D-Mannitol	0	0	0	−
D-Mannose	100	100	100	+
Melibiose	0	0	0	−
α-CH_3-glucoside	0	0	0	−
Raffinose	0	0	0	−
L-Rhamnose	0	0	0	−
Salicin	0	0	0	−
D-Sorbitol	0	0	0	−
Sucrose	9	36	91	+[6]
Trehalose	0	0	0	−
D-Xylose	0	0	0	−
Esculin hydrolysis	0	0	0	−
Acid from mucate	0	0	0	−
Tartrate (Jordan's)	73	91	91	+[2]
Acetate utilization	0	0	0	−
Lipase (corn oil)	0	0	0	−
DNase				
25°C	0	0	9	−
36°C	0	0	0	−
$NO_3^- \rightarrow NO_2^-$	100			+
Oxidase	0			−
ONPG	0	0	0	−
Citrate (Christensen's)	82	100	100	+
Red slant on LIA	55	64	64	+
Tyrosine clearing	100	100	100	+

[a] A blank space indicates that the test was not done at this time period.

[b] Symbols: −, negative at the end of appropriate incubation period; +, positive at 24 h or time of test. Superscript numbers indicate the day the reaction became positive.

RESULTS

DNA relatedness. Results from the previous study of Brenner et al. (2) indicated that seven strains of biogroup 3 were 81 to 99% related to the labeled DNA reference strain of biogroup 3 (0132-68) at 60°C (Table 3) but were only 44 to 49% related to strains of *P. alcalifaciens* biogroups 1 and 2, 26 to 33% related to strains of *P. stuartii*, and 32 to 34% related to strains of *Providencia (Proteus) rettgeri*. These data indicated that strains of biogroup 3 belonged to a new species.

Biochemical tests. Biochemically, strains of biogroup 3 were distinguished from other *Providencia* species because they produced acid from D-galactose but not from adonitol, *i*-inositol, or trehalose (Table 2). Eighty-two percent (9 of 11) of the strains produced gas from D-galactose within 7 days, 64% (7 of 11) produced gas from D-glucose, and 55% (5 of 11) produced gas from D-mannose. All of the strains produced gas from at least one of these three carbohydrates. Table 2 shows the tests that differentiated the four *Providencia* species in this revised classification.

Antibiotic susceptibility tests. The antibiotic susceptibilities of the 11 strains are shown in Table 5. Three strains, 9168-79, 9172-81, and

TABLE 5. Susceptibility of 11 *P. rustigianii* strains as determined by agar diffusion

Antibiotic[a]	Zone diameter (mm)			% Susceptible
	Range	Mean	SD	
Ampicillin (10)	6–27	21	5.7	91
Carbenicillin (100)	26–33	29	2.2	100
Cephalothin (30)	6–30	22	7.2	82
Chloramphenicol (30)	17–30	24	3.4	91
Colistin (10)	6–17	8	4.3	27
Gentamicin (10)	24–33	26	3.5	100
Kanamycin (30)	15–32	24	4.4	91
Nalidixic acid (30)	17–24	20	2.0	82
Penicillin (10 U)	6–18	11	3.6	0
Streptomycin (10)	10–22	18	3.5	82
Tetracycline (30)	14–24	21	3.5	82

[a] Figures in parentheses indicate the disk concentrations (in units for penicillin and in micrograms for all other antibiotics).

9174-81, were sensitive to colistin and also grew lighter than the other strains. None of the strains were sensitive to penicillin, but most were sensitive to the other antibiotics tested.

Description of *P. rustigianii* sp. nov. *P. rustigianii* sp. nov. is proposed for the group of strains previously known as biogroup 3 of *P. alcalifaciens* (4). The species name is treated as a neo (modern) Latin genitive noun, *rustigianii* (rus tig i an' i i, pronounced rus tidge ee ahn' ee eye), meaning "of Rustigian," in honor of Robert Rustigian, who did early studies on the *Proteus* group (7, 8). Strain ATCC 33673 (CDC no. 0132-68) is proposed as the type strain (holotype) for this species.

A complete description of *P. rustigianii* is given in Tables 1, 2, 4, and 5 *P. rustigianii* shares the general characteristics of the family *Enterobacteriaceae* (Table 2) and of the genus *Providencia*, which are as follows: positive tests for phenylalanine deaminase, acid production from D-mannose, tyrosine clearing, and indole production; resistance to colistin (8 of 11 strains); and negative tests for L-lysine and L-ornithine decarboxylase and L-arginine dihydrolase. *P. rustigianii* can be differentiated from other *Providencia* species because it produces acid from D-galactose but not from adonitol, *i*-inositol, and trehalose (Table 2).

DISCUSSION

The production of gas from D-glucose was one of the three tests used by Ewing et al. (4) to separate *P. alcalifaciens* and *P. stuartii* into biogroups (Table 1). *P. alcalifaciens* biogroups 1 and 2 differed phenotypically only in gas production, and Brenner et al. (2) showed by DNA relatedness that these two biogroups were members of the same species. *P. alcalifaciens* biogroup 3 (now *P. rustigianii*) and biogroup 4 (now *P. stuartii*) were also originally distinguished from each other only by gas production (biogroup 3 produced gas). The DNA relatedness data of Brenner et al. (2) showed biogroup 3 to be a separate species, distinct from *P. alcalifaciens* biogroups 1 and 2 and from *P. stuartii* biogroup 4. *P. rustigianii* (biogroup 3) strains produced gas from at least one carbohydrate, but not all strains produced a detectable amount of gas from D-glucose. It was thus difficult to distinguish strains of biogroups 3 and 4 until it was found that biogroup 3 was trehalose negative and biogroup 4 was trehalose positive (Table 1). In 1974, Ursing (9) showed that acid production from D-galactose was also useful for distinguishing the species of *Providencia*. We found that *P. alcalifaciens* (biogroups 1 and 2) did not produce acid from D-galactose, but *P. rustigianii* and *P. stuartii* (biogroups 4, 5, and 6) did (Table 1).

Of the 891 cultures of *Providencia* studied by Ewing et al. (4), 63% belonged in *P. alcalifaciens* biogroup 1, 9% belonged in *P. alcalifaciens* biogroup 2, and 27% belonged in *P. stuartii* biogroup 5. Only 2% were *P. rustigianii*, 2% were *P. stuartii* biogroup 4, and 3% were *P. stuartii* biogroup 6. Only 11 isolates of *P. rustigianii* were available for this study. Very little clinical information was given for these 11 isolates. Of the three sources known, all were from stool specimens. We hope this paper will stimulate others to search for this organism and determine its role in human disease.

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