Providencia friedericiana, a New Species Isolated from Penguins

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The name *Providencia friedericiana* sp. nov. is proposed for a well-defined group of organisms that were isolated from fecal specimens from penguins (*Aptenodytes patagonica*, *Eudyptes crestatus*, *Pyoscelis papua*, *Spheniscus demersus*, and *Spheniscus humboldti*). *P. friedericiana* strains are gram-negative, oxidase-negative, fermentative rods that grow on Endo agar, MacConkey agar, Wilson-Blair agar, and some other selective media for *Enterobacteriaceae*. They are acetate, adonitol, arginine dihydrolase, gelatinase, inositol, lactose, lysine decarboxylase, mannitol, ornithine decarboxylase, tartrate, trehalose, urease, and Voges-Proskauer negative, but fructose, galactose, glucose, glycerol, indole, catalase, KCN, nitrate reductase, mannose, methyl red, and phenylalanine deaminase positive. Simmons citrate is utilized weakly and variably. Gas is produced from glucose in small amounts or not at all. The organisms possess flagella when they are grown at 25°C. They show moderate motility at 25°C but are nonmotile or only slightly motile at 36°C. Capsules and slime layers are not produced. The type strain of *P. friedericiana*, strain 1/33, has been deposited with the Deutsche Sammlung von Mikroorganismen as strain DSM 2620.

The genus *Providencia*, which was described by Ewing in 1962, contains three species, *Providencia alcalifaciens*, *Providencia rettgeri*, and *Providencia stuartii*, which are included on the Approved Lists of Bacterial Names (17). From fecal specimens of penguins a well-defined group of bacteria that were distinguishable by their morphological and biochemical characteristics were isolated on media that are used for isolation of *Enterobacteriaceae*.

In this paper I describe the properties of 206 isolates belonging to 61 strains which resemble the members of the genus *Providencia*, yet differ from the three previously described species. This group most closely resembles *P. alcalifaciens* but differs from this species in producing negative acetate, adonitol, and tartrate reactions. Furthermore, citrate utilization, motility at 36°C, and gas production from glucose are weak or do not occur. Positive glycerol and sucrose reactions are delayed. There is evidence that these isolates constitute a new species. In this paper this new species is described, characterized, and classified. The name *Providencia friedericiana* sp. nov. is proposed for this organism.

MATERIALS AND METHODS

Source. A total of 206 isolates were obtained from feces of five different penguin species living in the zoological gardens of Duisburg, Frankfurt, Gelsenkirchen, Hamburg, Hannover, Cologne, Munich, and

Stuttgart, West Germany. Fecal samples from penguins from some other zoological gardens did not contain *P. friedericiana* isolates (Table 1).

Isolation. The fecal specimens were cultured on Endo agar, MacConkey agar, salmonella-shigella agar, and xylose-lysine-deoxycholate agar. Enrichment cultures in selenite and tetrathionate broth media were subcultured on these four plating media after overnight incubation at 36°C. Colonies of the new species formed on all media. Each colony that was different from the others was picked out and tested. A total of 61 strains were represented in the 206 isolates.

The following media and reagents were used for screening: indophenol-oxidase test medium, Kligler agar, lysine-indole motility medium (3), ornithine-indole motility medium (18), malonate-phenylalanine medium, Christensen urea agar, and Simmons citrate agar (8, 13).

Strains. Strains with typical reactions were studied by using additional biochemical tests. Type strains *P. alcalifaciens* ATCC 9886, *P. stuartii* (ATCC 25825, and *Proteus rettgeri* DSM 1131 (= NCTC 7481) were included for comparison.

Morphology. Colonial morphology and hemolysis of blood were determined on blood agar plates (blood agar base no. 2; Oxoid) containing 5% defibrinated sheep blood. The plates were incubated for 18 to 24 h at $36 \pm 1^\circ\text{C}$. Gram stains were performed by using 18- to 24-h cultures, as described elsewhere (11). Flagellar morphology was determined by the Forbes method (9) for 10 strains. For flagellum staining the bacteria were incubated on semisolid motility medium at 25 or 36°C for 18 to 48 h. The occurrence of a capsule was tested by the Duguid-Hiss method (10).

Biochemical tests. The biochemical tests were performed by using standard procedures (6, 8, 11, 13).

TABLE 1. *P. fredericiana* isolates obtained from penguins

Laboratory isolate(s)	Isolated from:	
	Animal no.	Species
1/1, ^a 1/2, 1/3, 1/4, 1/6, ^a 1/7, 1/8, 1/9, 1/10, 1/11, 1/12, 1/13, 1/15	1A	<i>Aptenodytes patagonica</i>
1/16, ^a 1/17	2A	<i>Spheniscus humboldti</i>
1/18, ^a 1/19, ^a 1/20, ^a 1/21	6A	<i>Spheniscus demersus</i>
1/22, ^a 1/23, 1/24, ^a 1/25, 1/26, 1/27, 1/28, 1/29, 1/30, 1/31	8A	<i>Aptenodytes patagonica</i>
1/32, ^a 1/33, ^a 1/34, ^a 1/35, 1/36, 1/37, 1/38, 1/39, 1/40, 1/41, ^a 1/42, 1/43, 1/44, 1/45, 1/46, 1/47	10A	<i>Pyoscelis papua</i>
1/48, ^a 1/49, 1/50, 1/51, 1/52, 1/53, 1/54	12A	<i>Aptenodytes patagonica</i>
1/55, ^a 1/56, ^a 1/57, 1/58, 1/59, 1/60, 1/61, 1/62, 1/63, ^a 1/64, ^a 1/65, ^a 1/66, ^a 1/67, 1/68, 1/69, 1/70, 1/71, 1/72, 1/73, 1/74	14A	<i>Pyoscelis papua</i>
1/75, ^a 1/76, ^a 1/77, 1/78, ^a 1/79, 1/80, 1/81, ^a 1/82, 1/83, 1/84	15A	<i>Pyoscelis papua</i>
1/86, ^a 1/87, 1/88, 1/90, 1/93, 1/94, 1/95, 1/96, 1/97, 1/98	16A	<i>Eudyptes crestatus</i>
1/99, ^a 1/100, 1/101	17A	<i>Spheniscus humboldti</i>
1/102, ^a 1/103, 1/106, 1/111, ^a 1/112, 1/113, 1/114, 1/115, 1/116, 1/117, 1/118	18A	<i>Aptenodytes patagonica</i>
1/119, ^a 1/120, ^a 1/121, 1/122, ^a 1/123, ^a 1/124, 1/125, 1/126, 1/127, 1/128, ^a 1/129, 1/130, 1/131, 1/132, 1/133 ^a	19A	<i>Aptenodytes patagonica</i>
1/134, ^a 1/135, ^a 1/136, 1/137 ^a	28A	<i>Spheniscus humboldti</i>
1/138, ^a 1/139, ^a 1/140, 1/141 ^a	31A	<i>Spheniscus demersus</i>
1/142, ^a 1/147 ^a	32A	<i>Spheniscus demersus</i>
1/149 ^a	33A	<i>Spheniscus demersus</i>
1/150, ^a 1/151, 1/152, 1/153 ^a	35A	<i>Aptenodytes patagonica</i>
1/143, ^a 1/144, ^a 1/145, ^a 1/146, 1/154, ^a 1/155, 1/156, 1/157, 1/158, 1/159	36A	<i>Aptenodytes patagonica</i>
2/110, ^a 2/155, ^a 2/159, ^a 2/195, 2/196, 2/202, 2/203, 2/204	9B	<i>Aptenodytes patagonica</i>
2/166, ^a 2/213, 2/219, 2/221, 2/223, 2/224, 2/225, 2/226, 2/227	14B	<i>Aptenodytes patagonica</i>
2/181, ^a 2/238, ^a 2/240, 2/241, 2/242, 2/263, ^a 2/264, ^a 2/318, ^a 2/327, ^a 2/329, 2/330, 2/331, 2/332, 2/335, 2/337, 2/341, 2/342	28B	<i>Pyoscelis papua</i>
2/343, ^a 2/344, 2/355, 2/372, ^a 2/373, 2/375, 2/376, 2/378, 2/379, 2/380, 2/382, 2/383	30B	<i>Eudyptes crestatus</i>
2/385, ^a 2/386, 2/387, 2/388, 2/392, 2/396, 2/400, 2/402, 2/403, 2/405, ^a 2/406, 2/407, 2/409, 2/412	31B	<i>Spheniscus humboldti</i>

^a Different strains from individual animals.

Pigment production. Pigment production was assayed on a nutrient agar containing 8 g of tyrosine per liter and 0.25 g of soluble ferric pyrophosphate per liter under aerobic conditions.

Guanine-plus-cytosine content. The guanine-plus-cytosine content of deoxyribonucleic acid was determined optically by thermal denaturation (R. Hammann, personal communication).

Antimicrobial agent susceptibility tests. Antibioassays were done on Mueller-Hinton agar by the disk method, as described by Bauer et al. (2). Zone sizes were designated as susceptible, intermediate, or resistant according to the recommendations of the DIN 58940 method (7).

RESULTS

Morphology. *P. fredericiana* cells were gram-negative, nonsporeforming, and rod shaped

(about 0.5 by 1.0 to 3.0 µm). The organisms grown at 25°C showed many peritrichously flagellated forms with five to eight flagella. However, when strains were grown at 36°C, they produced no or only a few flagellated cells; the flagellar morphology was poor, and the number of flagella was one or two. Capsules of slime layers were not detected.

Growth on media. Colonies of *P. fredericiana* grown overnight on blood agar plates at 36°C were about 1.0 to 2.0 mm in diameter, glossy, semitranslucent, and smooth. Hemolysis did not occur. The strains grew poorly at 4 and 44°C, moderately between 10 and 22°C, and well at 30, 36, and 40°C. There was also good growth on differential plating media that are selective for *Enterobacteriaceae*, with some exceptions (Ta-

TABLE 2. Growth and appearance of 19 *P. friedericiana* strains on selective plating media

Agar	Appearance of colonies after:		Growth of 19 strains after 1 day				Growth of 19 strains after 2 days			
	1 day	2 days	None	Poor	Medium	Good	None	Poor	Medium	Good
Brilliant green	Colorless	Greenish	1 ^a	18				15	4	
Cetrimide	Colorless	Colorless	14	5			7	12		
Endo	Pink	Red			19				19	
Eosine-methylene blue	Colorless	Pink			3	16			1	18
Hektoen	Green, orange, yellow	Green, orange, yellow				19				19
Leifson	Colorless	Yellow, brown		1	3	15			1	18
MacConkey	Orange, red	Orange, red				19				19
Salmonella-shigella	Colorless	Red, brown	3	16			17	2		
Thiosulfate-citrate-bile salt sucrose	Yellow	Yellow, green				19				19
Wilson-Blair	Yellow, green	Green, brown			19				3	16
Xylose-lysine deoxycholate	Yellow, pink	Yellow, pink			13	6			3	16

^a Number of strains.

ble 2). *P. friedericiana* produced a brownish pigment like other *Providencia* species and some *Legionella* species (1). Previously, Buttiaux, et al. noted that this pigmentation is produced only by members of the genus *Providencia* (5).

Biochemical reactions and description of *P. friedericiana*. The results of biochemical tests for 61 *P. friedericiana* strains and type strain 1/33 (= DSM 2620) are shown in Table 3. All strains were oxidase negative and catalase positive and reduced nitrate to nitrite; they showed the characteristics of the *Enterobacteriaceae*. Furthermore, these strains were negative in tests for arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase and positive for phenylalanine deaminase. They did not produce H₂S, hydrolyze urea, or utilize malonate. The strains gave weak reactions for Simmons citrate utilization. They produced indole after growth in tryptone broth, but the production of indole in lysine-indole motility medium or ornithine-indole motility medium was very weak. About one-half of the strains were nonmotile at 36°C; the others showed insignificant motility. Most strains were moderately motile at 25°C, as determined in semisolid motility medium containing 2,3,5-triphenyltetrazolium chloride. *P. friedericiana* fermented glucose with the production of acid; gas was produced, but only in small amounts (often about a pinhead-sized gas bubble in Durham tubes) by most strains. All strains fermented galactose, fructose, and mannose after 24 h and glycerol and sucrose after 48 h, but not adonitol, inositol, lactose, maltose, mannitol, trehalose, xylose, or some other carbohydrates. They gave negative Voges-Prosk-

kauer reactions and were positive in the methyl red test. Tyrosine clearing was positive. These organisms did not utilize acetate or Jordan tartrate.

Differentiation of *P. friedericiana* from other *Providencia* species. The uniform biochemical profile of the new group (Table 3) and its circumscribed habitat justify the proposition of a new species. This organism belongs to the family *Enterobacteriaceae* and to the genus *Providencia*. Table 4 shows tests that are useful in identifying *P. friedericiana*. This species is distinguishable from *P. alcalifaciens* by its regular negative reactions for acetate, adonitol, and tartrate, by its weak positive reactions for Simmons citrate utilization, by being nonmotile or very weakly motile at 36°C, by no gas production or weak gas production from glucose, and by its obligately delayed positive reactions for glycerol and sucrose. In individual cases, however, differentiation between *P. friedericiana* and *P. alcalifaciens* may be difficult.

The description of *P. alcalifaciens* by Edwards and Ewing was based on an investigation of 633 strains (8). Of these strains, 83.9% belonged to biogroup 1, 10.9% belonged to biogroup 2, 2.5% belonged to biogroup 3, and 2.7% belonged to biogroup 4. The members of biogroups 3 and 4 did not ferment either adonitol or inositol. Therefore, 5% of these *P. alcalifaciens* strains resemble *P. friedericiana* more or less. Unfortunately, Edwards and Ewing did not describe the distribution of other biochemical reactions in the four biogroups, with the exception of gas production and fermentation of adonitol and inositol. Tartrate utilization is the single differ-

TABLE 3. Biochemical reactions of *P. fredericiana* strains and the type strain

Test	No. of strains tested	Cumulative % of strains positive after:		Reaction of type strain 1/33 (= DSM 2620)
		1 to 2 days	3 to 7 days	
Acetate utilization	61	0	0	—
Acid from adonitol	48	0	0	—
Acid from L-arabinose	61	0	0	—
Arginine dihydrolase	61	0	0	—
Catalase production	61	100	100	+
Acid from cellobiose	29	0	0	—
Chitinase production	48	0	0	—
Citrate (Christensen) utilization	61	100	100	+
Citrate (Simmons) utilization	61	70 ^a	90 ^a	+ ^a
Collagenase	48	0	0	—
Deoxyribonuclease production	48	0	0	—
Acid from dulcitol	48	0	0	—
Acid from erythritol	29	0	0	—
Esculin hydrolysis	48	0	0	—
Acid from D-fructose	48	98	98	+
Acid from D-galactose	48	98	98	+
Gelatin hydrolysis	48	0	0	—
Acid from D-glucose	61	100	100	+
Gas from D-glucose	61	49 ^a	90 ^a	+ ^a
Acid from glycerol	48	90	100	+
Acid from glycogen	48	0	0	—
Hemolysis on sheep blood agar	61	0	0	—
H ₂ S production (Kligler)	61	0	0	—
Indole production	61	100	100	+
Acid from <i>m</i> -inositol	48	0	0	—
Acid from inulin	48	0	0	—
Growth in KCN	48	98	100	+
Acid from D-lactose	61	0	0	—
Lecithinase production	48	0	0	—
Lipase (Tween 80 hydrolysis)	48	0	0	—
Lysine decarboxylase production	61	0	0	—
Utilization of malonate	61	0	0	—
Acid from maltose	61	0	0	—
Acid from D-mannitol	61	2	2	—
Acid from D-mannose	48	100	100	+
Acid from melezitose	48	0	0	—
Acid from melibiose	48	0	0	—
Methyl red (36°C)	29	100	100	+
Motility at 25°C	61	87 ^a	89 ^a	+ ^a
Motility at 36°C	61	48 ^a	51 ^a	+ ^a
Mucate utilization	29	0	0	—
Nitrite from nitrate	48	100	100	+
ONPG production ^b	48	2	—	—
Ornithine decarboxylase production	61	0	0	—
Oxidase production	61	0	0	—
Pectate utilization	29	0	0	—
Phenylalanine deaminase production	61	100	100	+
Pigment brownish around the colonies in the presence of iron ions, oxygen, and tyrosine	29	100	100	+
Acid from raffinose	48	0	0	—
Acid from L-rhamnose	61	8	8	—
Hydrolysis of salicin	29	0	0	—
Acid from sorbitol	48	0	0	—
Acid from L-sorbose	48	0	0	—
Hydrolysis of starch	48	0	0	—
Acid from sucrose	48	8	100	+ ^c
Tartrate (Jordan) utilization	48	0	0	—
Acid from trehalose	61	0	0	—
L-Tyrosine clearing	13	100	100	+
Urease production	61	0	0	—
Voges-Proskauer reaction	13	0	0	—
Acid from D-xylose	61	0	0	—

^a Weak reaction.^b ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.^c Delayed reaction.

TABLE 4. Tests of value in differentiating *P. fredericiana* from other *Providencia* species

Test	<i>P. fredericiana</i>		<i>P. alcalifaciens</i>						<i>P. stuartii</i>				<i>P. rettgeri</i>	
	% Positive	Reaction of strain 1/33 ^T	% Positive (reference 4) ^a	% Positive (reference 8) ^b	Reaction of:				% Positive (reference 4) ^a	% Positive (reference 8) ^b	Reaction of:		% Positive (reference 4) ^a	% Positive (reference 8) ^b
					Bio-group 1	Bio-group 2	Bio-group 3	Bio-group 4			Bio-group 5	Bio-group 6		
Acetate utilization	0/0 ^c	—	30	27/29 ^d					81	84/8 ^d			59	59/23 ^d
Acid from adonitol	0/0	—	94	95/0.2	+	+	—	—	4	4	—	+	99	81/6
Citrate (Simmons)	70/90 ^e	+ ^e	98	98/1.3					93	96/3			96	96/8
Gas from glucose	49/90 ^e	+ ^e	85	86/0.6	+	—	+	—	0	0	—	—	12	12
Acid from glycerol	90/100	+	12	11/55					12	34/66			66	66/33
Acid from <i>m</i> -inositol	0/0	—	1	1	—	—	—	—	97	98/3	+	+	93	93/5
Acid from mannitol	2/2	—	2	2/0.2					13	13/1			99	89
Motility at 36°C	48/51 ^e	+ ^e	96	96/0.3					86	86/3			94	94
Acid from sucrose	8/100	+ ^f	13	13/74					31	26/66			13	13/57
Tartrate (Jordan)	0/0	—	100	100	+	+	+	+	96	96			96	96
Acid from trehalose	0/0	—	4	4/1					99	87/6			1	16/1
Urease production	0/0	—	0	0	—	—	—	—	15	0			99	100

^a Percentage of strains positive after 2 days; data from the study of Brenner et al. (4).

^b Data from the study of Edwards and Ewing (8).

^c Cumulative percentage of strains positive after 1 to 2 days/cumulative percentage of strains positive after 3 to 7 days.

^d Percentage of strains positive after 2 days/percentage of strains positive after 3 or more days.

^e Weak reaction.

^f Delayed reaction.

ence between *P. alcalifaciens* biogroup 3 strains and *P. friederician*a. However, biogroup 3 strains do not belong to the other groups (e.g., biogroups 1 and 2), as Brenner et al. have shown (4), and all biogroup 4 strains examined have shown the biochemical characteristics of *P. stuartii*. Therefore, some strains that have been described previously as *P. alcalifaciens* may belong to the new species *P. friederician*a, which shows a clear-cut biochemical profile with less variability.

*P. friederician*a is distinguished from *P. stuartii* on the basis of its negative reactions for acetate, inositol, Jordan tartrate, and trehalose and its positive reactions for glycerol and sucrose. Negative acetate, adonitol, inositol, manitol, Jordan tartrate, and urease tests differentiate *P. friederician*a from *P. rettgeri*.

Guanine-plus-cytosine content. The guanine-plus-cytosine contents of the deoxyribonucleic acids of strains 1/1 and 1/33^T (T = type strain) were determined spectrophotometrically by thermal denaturation. The ratios obtained were 39 ± 1.5 mol%, corresponding well to the values determined previously for the genus *Providencia* (4, 12).

Antimicrobial susceptibility. Eight strains and a total of 18 cultures were tested for susceptibility to amikacin, ampicillin, azlocillin, cefalotin, cefazolin, cefotaxim, cefoxitin, cefuroxim, chloramphenicol, clindamycin, erythromycin, fosfomycin (plus glucose 6-phosphate), fusidic acid, gentamicin, mezlocillin, nitrofurantoin, oxacillin, penicillin G, pipemidic acid, piperacillin, tetracycline, tobramycin, and trimethoprim-sulfamethoxazole. All strains were resistant to clindamycin, fusidic acid, penicillin G, and oxacillin and showed intermediate susceptibility to nitrofurantoin. Seven strains were resistant to erythromycin, and one showed intermediate susceptibility. Six strains were susceptible to ampicillin, one was resistant, and one showed intermediate susceptibility. Seven strains were susceptible to cephalotin and chloramphenicol, and one was intermediately susceptible. Full susceptibility to all other substances was observed. There was no clear distinction between *P. friederician*a and the strains of *P. alcalifaciens* and *P. stuartii* tested.

DISCUSSION

Their growth characteristics, guanine-plus-cytosine contents, and biochemical profiles indicate that the strains isolated from penguins belong to the genus *Providencia*. The common habitat and the occurrence of the organisms in the intestinal tracts of different penguin species suggest a genetically and phenotypically homogeneous group. A number of phenotype features

show that these bacteria are different from the previously described *Providencia* species. However, the lack of detail in the description of *P. alcalifaciens* creates some difficulties. Of course, Brenner et al. (4) found that the eight biogroup 3 strains which they studied belonged to a hybridization group that was separate from the strains of biogroups 1 and 2 and suggested that biogroup 3 strains represent a new species. Unfortunately, these authors studied no strains of biogroup 4 because all of the strains presumed to belong to biogroup 4 were typical strains of *P. stuartii*. It is possible that real strains of biogroup 4 are anaerogenic strains of *P. friederician*a. Therefore, the suggestion of Brenner et al. endorses the proposal to establish *P. friederician*a as a new species with a circumscribed habitat.

There is some evidence that bacterial taxa are as old as the ecological niches in which they are living (15, 16). Therefore, *P. friederician*a may have existed as a normal inhabitant of the intestines of penguins since the Tertiary period. The habitats of the other *Providencia* species are not known (12, 13); it is possible that these organisms were derived from *P. friederician*a by acquisition of properties that improved their environmental survival. Greater mobility, utilization of acetate and tartrate, and stronger utilization of citrate may be viewed as adaptations to the outer world. An alternative hypothesis is that *P. friederician*a descended from *P. alcalifaciens* by loss of the ability to utilize some compounds, regular fermentation of some other carbohydrates, and reduced motility at 36°C, as it occurs in numerous species and is well adapted to life in its hosts.

The very few biogroup 3 and 4 strains of *P. alcalifaciens* that have been isolated from humans seem to be atypical strains of *P. friederician*a as indicated by positive tartrate utilization. The other biochemical properties of these biogroups have not been described with sufficient precision.

Description of *Providencia friederician*a sp. nov. This closed group of organisms from a well-defined habitat without intimate contact with human beings suggests the establishment of a new independent species within the genus *Providencia*. Therefore, I propose the name *Providencia friederician*a sp. nov. (frie.de.ri.ci.a'na. M.L. adj. *friederician*a, derived from the German names Friederike and Friederich [most of the technical work of this study was done by Friederike Heimbach]). Strain 1/33 (= DSM 2620) is the type strain of the species.

Motile by peritrichous flagella at 25°C; motility mostly absent at 36°C. Not encapsulated. Brownish pigment produced under special conditions. The biochemical properties are shown in

Table 3. Tests useful for differentiating *P. friedericiana* from other *Providencia* species are shown in Table 4.

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ADDENDUM IN PROOF

Recently, Hickman-Brenner et al. (F. W. Hickman-Brenner, J. J. Farmer III, A. G. Steigerwalt, and D. J. Brenner, *J. Clin. Microbiol.* 17:1057-1060, 1983) described *Providencia rustigiana* as a new species in the family *Enterobacteriaceae*. It was formerly known as *Providencia alcalifaciens* biogroup 3. The biochemical properties of the 11 studied strains seem to be very similar to those of *P. friedericiana*.

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