Providencia heimbachae, a New Species of Enterobacteriaceae Isolated from Animals

H. E. MÜLLER,1 C. M. O'HARA,2* G. R. FANNING,4 F. W. HICKMAN-BRENNER,3 J. M. SWENSON,2 AND DON J. BRENNER3

Staatliches Medizinaluntersuchungsamt, Braunschweig, Federal Republic of Germany; 
Hospital Infections Program2 and Division of Bacterial Diseases,3 Centers for Disease Control, Atlanta, Georgia 30333; and Walter Reed Army Institute of Research, Washington, D.C. 20307

The name Providencia heimbachae sp. nov. is proposed for a group of organisms that were isolated from the feces of penguins in the Federal Republic of Germany and from a cow in the United States. P. heimbachae strains are gram-negative, oxidase-negative, fermentative, rod-shaped organisms that grow on MacConkey agar, as well as other media that are selective for members of the Enterobacteriaceae. The 13 P. heimbachae isolates which we studied gave negative results for the following tests: indole production, Voges-Proskauer, Simmons citrate, H2S production on triple sugar iron and Kligler agars, urease, lysine decarboxylase, arginine dihydrolase, gelatinase, growth in the presence of KCN, malonate, acid production from L-arabinose, cellobiose, dulcitol, erythritol, lactose, melezitose, melibiose, α-methylglucoside, raffinose, salicin, D-sorbitol, L-sorbose, starch, D-tagatose, trehalose, D-turanose, and D-xylOSe (15% positive after 7 days), esculin hydrolysis, acetate, lipase, deoxyribonuclease, and α-nitrophenyl-β-D-galactopyranoside. The strains were positive for the following tests: catalase, nitrate reductase, methyl red, phenylalanine deaminase, acid production from D-glucose, adonitol, D-arabitol, D-fructose, D-galactose, D-mannose, and L-rhamnose, and tyrosine clearing. Acid production from glycerol, myo-inositol, maltose, and D-mannitol was delayed; gas was produced from D-glucose, D-galactose, and D-mannose in small amounts or not at all (54% positive after 7 days). Motility at 36°C was variable (46% positive after 2 days; 85% positive after 7 days). Deoxyribonucleic acids from 12 other strains of P. heimbachae were highly related (91 to 100% related in reactions assayed on hydroxyapatite at 60 and 75°C) to 32P-labeled deoxyribonucleic acid from the proposed type strain (strain ATCC 35613). Labeled deoxyribonucleic acid from this type strain was 22 to 45% related to 32P-labeled deoxyribonucleic acid from four other Providencia species. The levels of relatedness of P. heimbachae to Proteus species ranged from 10 to 13% in 60°C reactions. P. heimbachae could be differentiated from the other Providencia species by the following characteristics: negative tests for Simmons citrate, urease, indole production, and acid production from trehalose and positive tests for acid production from adonitol, D-arabitol, D-galactose, and L-rhamnose. All of the P. heimbachae strains which we studied were resistant to tetracycline, and most strains were resistant to cephalothin.

In 1983, a new Providencia species was described independently by workers in two laboratories under the names Providencia rustigianii (9) and Providencia friedericiana (12). Deoxyribonucleic acid (DNA) hybridization studies on the type strains and other strains have shown that these two groups are the same species (Hickman-Brenner, Fanning, Müller, and Brenner, manuscript in preparation). However, some strains that were formerly considered a biogroup of Providencia friedericiana differed from Providencia rustigianii both biochemically and as determined by DNA relatedness. Strain CDC 1519-73 (Table 1), with the vernacular name “Enteric Group 78,” was also found to belong to this biogroup.

In this study we characterized this new group of Providencia by using biochemical and DNA hybridization tests, as well as antimicrobial agent susceptibility tests. On the basis of these data, the name Providencia heimbachae sp. nov. is proposed, and strain MUA 2-110 (= CDC 8025-83 = ATCC 35613) is designated the type strain.

MATERIALS AND METHODS

Bacterial strains. The 13 strains of Providencia heimbachae which we studied and their sources are shown in Table 1. All of the strains were maintained on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) or tryptone soy agar (Oxoid USA, Columbia, Md.) at room temperature (18 to 28°C), and all incubations took place at 36 ± 1°C unless indicated otherwise. The other strains used in this study (Table 2) have been described previously (8).

Media and biochemical tests. Commercial media were used whenever possible. The biochemical tests (Table 3) were performed by using standardized procedures (3–9, 11). The tests for Simmons citrate utilization, growth in Moeller KCN broth, and production of indole were standardized and were performed exactly as described by Edwards and Ewing (4) and Farmer et al. (5).

Pigment production. Pigment production was assayed on casein hydrolysate or yeast extract agar containing 10 g of either L-histidine, L-phenylalanine, L-tryptophan, or L-tyrosine per liter of medium (pH 7.4). A 10-g/liter portion of CaCO₃ powder (to improve recognition of pigment production by providing a whitish background) and 0.25 g/liter of a ferric pyrophosphate solution (filter sterilized) were added aseptically after the other ingredients had been autoclaved. The tests were done aerobically.

Antimicrobial agent susceptibility tests. Antimicrobial agent susceptibility (Table 4) was determined by broth microdilution and disk diffusion methods, using the standardized methods described by the National Committee for
Clinical Laboratory Standards (14, 15). The drugs tested were supplied by the manufacturers. Ampicillin, carbenicillin, piperacillin, mezlocillin, ticaricillin, cephalothin, cefazolin, cefamandole, cefoxitin, cefuroxime, cefoperazone, cefotaxime, cefadiazime, moxalactam, ceftriaxone, gentamicin, amikacin, tobramycin, netilmicin, tetracycline, sulfamethoxazole-trimethoprim, sulfisoxazole, trimethoprim, and chloramphenicol were tested by both methods. Azlocillin, doxycline, nalidixic acid, cinoxacin, nitrofurantoin, imipenem, and aztreonam were tested by the broth microdilution method only.

**DNA relatedness.** Levels of DNA relatedness were determined by reacting 
\(^{32}\)P-labeled DNA from *Providencia heimbachae* 8025-83\(^T\) (T = type strain) with unlabeled DNAs from the 12 other *Providencia heimbachae* strains and other strains of interest (Table 2), using methods described elsewhere (2, 9, 10).

**RESULTS AND DISCUSSION**

**Isolation of bacterial strains.** Twelve of the *Providencia heimbachae* strains were isolated from the feces of apparently healthy penguins, and one strain was isolated from an aborted bovine fetus (Table 1). The habitat of the new species seems to be the same as or similar to the habitat of *Providencia rustigianii*, and *Providencia heimbachae* has been isolated frequently from the same animal sources, although it has not yet been isolated from humans. Its estimated occurrence in relation to that of *Providencia rustigianii* is 1:10, which corresponds to the numbers isolated from penguins (12) as well as to the numbers of strains in the collection at the Centers for Disease Control (9).

**TABLE 1. List of *Providencia heimbachae* strains studied**

<table>
<thead>
<tr>
<th>Strain(^a)</th>
<th>Species of penguin</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDC 8025-83(^T)</td>
<td><em>Aptenodytes patagonica</em></td>
<td>Feces</td>
</tr>
<tr>
<td>(= MUA 2-110(^T),</td>
<td></td>
<td></td>
</tr>
<tr>
<td>= ATCC 35613(^T)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC 8027-83</td>
<td><em>Aptenodytes patagonica</em></td>
<td>Feces</td>
</tr>
<tr>
<td>(= MUA 1-152)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC 8032-83</td>
<td><em>Spheniscus humboldti</em></td>
<td>Feces</td>
</tr>
<tr>
<td>(= MUA 1-154)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC 8035-83</td>
<td><em>Spheniscus humboldti</em></td>
<td>Feces</td>
</tr>
<tr>
<td>(= MUA 1-134)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC 8039-83</td>
<td><em>Spheniscus demersus</em></td>
<td>Feces</td>
</tr>
<tr>
<td>(= MUA 1-140)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC 8050-83</td>
<td><em>Spheniscus humboldti</em></td>
<td>Feces</td>
</tr>
<tr>
<td>(= MUA 1-135)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC 8051-83</td>
<td><em>Spheniscus humboldti</em></td>
<td>Feces</td>
</tr>
<tr>
<td>(= MUA 1-136)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC 8052-83</td>
<td><em>Spheniscus demersus</em></td>
<td>Feces</td>
</tr>
<tr>
<td>(= MUA 2-138)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC 8053-83</td>
<td><em>Spheniscus demersus</em></td>
<td>Feces</td>
</tr>
<tr>
<td>(= MUA 1-139)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC 8054-83</td>
<td><em>Aptenodytes patagonica</em></td>
<td>Feces</td>
</tr>
<tr>
<td>(= MUA 1-150)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC 8055-83</td>
<td><em>Aptenodytes patagonica</em></td>
<td>Feces</td>
</tr>
<tr>
<td>(= MUA 1-151)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC 8056-83</td>
<td><em>Aptenodytes patagonica</em></td>
<td>Feces</td>
</tr>
<tr>
<td>(= MUA 2-202)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC 1519-73</td>
<td>Aborted bovine fetus</td>
<td></td>
</tr>
<tr>
<td>(enteric group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(78)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) CDC, Centers for Disease Control, Atlanta, Ga.; MUA, Staatliches Medizinaluntersuchungsamt, Braunschweig, Federal Republic of Germany; ATCC, American Type Culture Collection, Rockville, MD.

**TABLE 2. Levels of DNA relatedness of *Providencia heimbachae* ATCC 35613\(^T\) to 12 other *Providencia heimbachae* strains and strains belonging to other species**

<table>
<thead>
<tr>
<th>Source of unlabeled DNA</th>
<th>Relative binding ratio at 60°C (%)(^b)</th>
<th>Divergence (%)(^c)</th>
<th>Relative binding ratio at 75°C (%)(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Providencia heimbachae</em> ATCC 35613(^T)</td>
<td>100</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td><em>Providencia heimbachae</em> 8027-83</td>
<td>100</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td><em>Providencia heimbachae</em> 8039-83</td>
<td>100</td>
<td>1.0</td>
<td>99</td>
</tr>
<tr>
<td><em>Providencia heimbachae</em> 8050-83</td>
<td>100</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td><em>Providencia heimbachae</em> 8051-83</td>
<td>100</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td><em>Providencia heimbachae</em> 8052-83</td>
<td>100</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td><em>Providencia heimbachae</em> 8053-83</td>
<td>100</td>
<td>0.0</td>
<td>99</td>
</tr>
<tr>
<td><em>Providencia heimbachae</em> 8054-83</td>
<td>100</td>
<td>0.0</td>
<td>99</td>
</tr>
<tr>
<td><em>Providencia heimbachae</em> 8055-83</td>
<td>100</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td><em>Providencia heimbachae</em> 8056-83</td>
<td>99</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td><em>Providencia heimbachae</em> 8032-83</td>
<td>99</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td><em>Providencia heimbachae</em> 8035-83</td>
<td>91</td>
<td>0.0</td>
<td>ND(^e)</td>
</tr>
<tr>
<td><em>Providencia rustigianii</em> 0132-68(^T)</td>
<td>45</td>
<td>6.5</td>
<td>31</td>
</tr>
<tr>
<td><em>Providencia alcalifaciens</em> 3370-67</td>
<td>28</td>
<td>14.5</td>
<td>ND</td>
</tr>
<tr>
<td><em>Providencia rettgeri</em> 1163(^T)</td>
<td>24</td>
<td>13.5</td>
<td>6</td>
</tr>
<tr>
<td><em>Providencia stuartii</em> 2896-68</td>
<td>22</td>
<td>15.0</td>
<td>4</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em> PR-14</td>
<td>13</td>
<td>15.0</td>
<td>ND</td>
</tr>
<tr>
<td><em>Proteus myxofaciens</em> ATCC 19692(^T)</td>
<td>11</td>
<td>16.0</td>
<td>ND</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em> PR-1</td>
<td>10</td>
<td>16.0</td>
<td>ND</td>
</tr>
<tr>
<td><em>Morganella morganii</em> ATCC 25830(^T)</td>
<td>9</td>
<td>14.5</td>
<td>ND</td>
</tr>
<tr>
<td><em>Escherichia coli</em> K-12</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^a\) Labeled DNA was reacted with unlabeled DNA from the same strain (homologous reaction) and with DNAs from other strains (heterologous reactions). Each reaction was done at least twice. Before normalization, the level of reassociation in homologous reactions averaged 55%. Control reactions, in which labeled DNA was incubated in the absence of unlabeled DNA, resulted in 0.5 to 1.5% binding to hydroxyapatite. These control values were subtracted before normalization.

\(^b\) Relative binding ratios were determined as follows: [percentage of DNA bound to hydroxyapatite in heterologous reaction]/[percentage of DNA bound to hydroxyapatite in homologous reaction] × 100.

\(^c\) Divergence was calculated on the assumption that a 1% decrease in the thermal stability of a heterologous DNA duplex compared with the thermal stability of the homologous DNA duplex was caused by 1% of the bases within the duplex that were unpaired; divergence was calculated to the nearest 0.5%.

\(^d\) ND, Not determined.

DNA hybridization. Labeled DNA from the type strain of *Providencia heimbachae* was 91 to 100% related to DNAs from the 12 other *Providencia heimbachae* strains in DNA relatedness reactions done at 60°C. There was 0 to 0.5%
TABLE 3. Biochemical reactions of 13 Providencia heimbachae strains and type strain ATCC 35613

<table>
<thead>
<tr>
<th>Test</th>
<th>Reaction of type strain ATCC 35613</th>
<th>Cumulative % positive after:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 Day</td>
</tr>
<tr>
<td>Indole*</td>
<td>ND*</td>
<td>0</td>
</tr>
<tr>
<td>Methyl red</td>
<td>ND</td>
<td>85</td>
</tr>
<tr>
<td>Voges-Prokauer</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>Citrate (Simmons)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H₂S production on Kliger or triple sugar iron agar</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Urea</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>100</td>
<td>ND</td>
</tr>
<tr>
<td>Lysine (Moeller)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arginine (Moeller)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ornithine (Moeller)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Motility</td>
<td>15</td>
<td>46</td>
</tr>
<tr>
<td>Gelatin (22 and 36°C)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Growth in the presence of KCN*</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Malonate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D-Glucose, acid</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Gas</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acid from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adonitol</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D-Arabitol</td>
<td>62</td>
<td>92</td>
</tr>
<tr>
<td>Cellobiotose</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dulcitol</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erythritol</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fructose*</td>
<td>92</td>
<td>100</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>Glycerol</td>
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<td>0</td>
</tr>
<tr>
<td>Glycogen</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>8</td>
<td>46</td>
</tr>
<tr>
<td>Inulin*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lactose</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maltose</td>
<td>8</td>
<td>54</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Salicin</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D-Sorbitol</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-Sorbitol*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Starch*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tagatose*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trehalose</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Turanose*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Esculin hydrolysis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acid from mucate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tartrate (Jordan)*</td>
<td>23</td>
<td>69</td>
</tr>
<tr>
<td>Acetate utilization</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lipase (corn oil and Tween 80)*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Deoxyribonuclease</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NO₃⁻ → NO²⁻</td>
<td>100</td>
<td>ND</td>
</tr>
<tr>
<td>Oxidase</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Catalase*</td>
<td>100</td>
<td>ND</td>
</tr>
<tr>
<td>o-Nitrophenyl-β-D-galactopyranoside</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Citrate (Christensen)</td>
<td>39</td>
<td>92</td>
</tr>
<tr>
<td>Tyrosine clearing</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*ND, Not done.
*+ and - indicate the end of the incubation period; +, positive after 24 h or at the time of the test. The numbers in parentheses indicate the day on which the reaction became positive.

TABLE 4. Ranges of MICs and categories of susceptibility as determined by the disk diffusion method for strains of Providencia heimbachae

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC range (µg/ml)</th>
<th>No. of strains in the following disk diffusion categories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Amoxicillin*</td>
<td>≤0.25-16</td>
<td>10</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>≤4</td>
<td>13</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>≤1.0</td>
<td>13</td>
</tr>
<tr>
<td>Mezlocillin</td>
<td>≤1.0</td>
<td>13</td>
</tr>
<tr>
<td>Azlocillin*</td>
<td>≤1.0-16</td>
<td>NT*</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>≤1.0</td>
<td>13</td>
</tr>
<tr>
<td>Cephalothin*</td>
<td>4-32</td>
<td>10</td>
</tr>
<tr>
<td>Cefazolin*</td>
<td>≤0.25-32</td>
<td>12</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>≤0.25</td>
<td>13</td>
</tr>
<tr>
<td>Cefoxitin*</td>
<td>0.5-4</td>
<td>11</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>≤0.25-2</td>
<td>13</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>≤0.5-1.0</td>
<td>13</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≤0.25</td>
<td>12</td>
</tr>
<tr>
<td>Cefazidine</td>
<td>≤1.0</td>
<td>13</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤0.5-1.0</td>
<td>13</td>
</tr>
<tr>
<td>Amikacin</td>
<td>≤0.5-4</td>
<td>13</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>≤0.25-2</td>
<td>13</td>
</tr>
<tr>
<td>Netilimicin</td>
<td>≤0.25-1.0</td>
<td>13</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>8-32</td>
<td>NT</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≥32</td>
<td>13</td>
</tr>
<tr>
<td>Sulfamethoxazole-trimethoprim*</td>
<td>≤1.20/0.06-2.4/0.12</td>
<td>13</td>
</tr>
<tr>
<td>Sulfisoxazole</td>
<td>≤4-64</td>
<td>13</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>≤1.0</td>
<td>13</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>1.0-4</td>
<td>NT</td>
</tr>
<tr>
<td>Cinoxacin</td>
<td>2-8</td>
<td>NT</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>32-64</td>
<td>NT</td>
</tr>
<tr>
<td>Moxalactam</td>
<td>≤0.25</td>
<td>13</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤0.5-1.0</td>
<td>13</td>
</tr>
<tr>
<td>Chloramphenicol*</td>
<td>8-16</td>
<td>7</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.25-1.0</td>
<td>NT</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>≤0.25</td>
<td>NT</td>
</tr>
</tbody>
</table>

*The following discrepancies between disk diffusion and MIC results were found: for amoxicillin, three minor discrepancies; for cefalothin, one major and one minor; for cefazolin, one very major and one minor; for cefotaxim, one major; and for chloramphenicol, three minor discrepancies. See text.

*NT, Not tested by the disk diffusion method.

We tested a solution containing 19 parts of sulfamethoxazole to 1 part of trimethoprim.

Divergence in the related DNA sequences, and the levels of relatedness were as high as 75°C reactions as in 60°C reactions (Table 2). Providencia heimbachae was 22 to 45% related to the other four species of Providencia, 97 to 13% related to Proteus species, 9% related to Morganella morgani, and 5% related to Escherichia coli. Thus, Providencia heimbachae is a distinct species that phenotypically and genetically belongs in the genus Providencia.

Antimicrobial agent susceptibility. Table 4 shows the minimal inhibitory concentration (MIC) ranges and the categorization.
TABLE 5. Tests useful for differentiating Providencia heimbachae from other Providencia species* 

<table>
<thead>
<tr>
<th>Test</th>
<th>Providencia heimbachae</th>
<th>Providencia rustigianii</th>
<th>Providencia retgeri</th>
<th>Providencia alcalfaciens</th>
<th>Providencia stuartii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole*</td>
<td>- (0)*</td>
<td>+ (100)</td>
<td>+ (99)</td>
<td>+ (99)</td>
<td>+ (98)</td>
</tr>
<tr>
<td>Citrate</td>
<td>- (0)</td>
<td>V (15)</td>
<td>+ (95)</td>
<td>+ (98)</td>
<td>+ (93)</td>
</tr>
<tr>
<td>Urea</td>
<td>- (0)</td>
<td>- (0)</td>
<td>+ (98)</td>
<td>- (0)</td>
<td>V (30)</td>
</tr>
<tr>
<td>Motility</td>
<td>V (46)</td>
<td>V (28)</td>
<td>+ (94)</td>
<td>+ (96)</td>
<td>V (85)</td>
</tr>
<tr>
<td>Growth in the presence of KCN</td>
<td>- (7)</td>
<td>+ (100)</td>
<td>+ (97)</td>
<td>+ (100)</td>
<td>+ (100)</td>
</tr>
<tr>
<td>Gas from D-glucose</td>
<td>- (0)</td>
<td>V (36)</td>
<td>- (10)</td>
<td>V (85)</td>
<td>- (0)</td>
</tr>
<tr>
<td>Acid from:</td>
<td>Adonitol</td>
<td>+ (92)</td>
<td>+ (100)</td>
<td>+ (98)</td>
<td>- (5)</td>
</tr>
<tr>
<td></td>
<td>D-Arabitol</td>
<td>+ (92)</td>
<td>+ (100)</td>
<td>- (0)</td>
<td>- (0)</td>
</tr>
<tr>
<td></td>
<td>D-Galactose</td>
<td>+ (92)</td>
<td>+ (96)</td>
<td>- (0)</td>
<td>+ (92)</td>
</tr>
<tr>
<td></td>
<td>i-Inositol</td>
<td>V (46)</td>
<td>+ (90)</td>
<td>- (1)</td>
<td>+ (95)</td>
</tr>
<tr>
<td></td>
<td>D-Mannitol</td>
<td>- (0)</td>
<td>+ (100)</td>
<td>- (2)</td>
<td>- (10)</td>
</tr>
<tr>
<td></td>
<td>L-Rhamnose</td>
<td>+ (100)</td>
<td>V (70)</td>
<td>- (0)</td>
<td>+ (98)</td>
</tr>
<tr>
<td></td>
<td>Trehalose</td>
<td>- (0)</td>
<td>- (0)</td>
<td>- (2)</td>
<td>- (0)</td>
</tr>
</tbody>
</table>

* All data except the data for Providencia heimbachae were obtained from reference 6.
* See text.
* +, 90% or more of the strains positive; V, 11 to 89% of the strains positive; -, 10% or less of the strains positive. All tests were performed at 36 ± 1°C. The numbers in parentheses are percentages of strains that were positive after 2 days.
* Minimal gas production.

rieties of susceptibility as determined by the disk diffusion method. The discrepancies that occurred between zone size and MIC were designated very major, major, or minor errors (very major, susceptible by zone size but resistant by MIC; major, resistant by zone size but susceptible by MIC; minor, intermediate or moderate susceptibility as determined by one method). Very major errors occurred for one isolate with ampicillin; major or minor errors occurred with ampicillin, cefazolin, cefotaxim, ceftriaxone, and chloramphenicol. Tetracycline, cefalothin, ampicillin, cefoxitin, and cefotaxim, and chloramphenicol were the least active drugs, but the remaining drugs tested had good activities. All of the strains were resistant to tetracycline, and 10 of the 13 strains were resistant to cefalothin. Single strains were resistant to ampicillin, cefazolin, and cefotaxim (Table 4).

Description of Providencia heimbachae sp. nov. For the new species described here we propose the name Providencia heimbachae (heimbachae, M. L. gen. fem. n. heimbachae of Heimbach, to honor Friederike Heimbach, who isolated 12 of the strains).

Strains of Providencia heimbachae are gram-negative, oxidase-negative, catalase-positive, D-glucose-fermenting, nitrate-reducing, straight rod-shaped organisms that conform to the definitions of the family Enterobacteriaceae (1) and the genus Providencia (16). They grow on Endo agar, MacConkey agar, and other similar selective media. Like members of other species of Providencia, strains of Providencia heimbachae (particularly fresh isolates) develop a red to brown pigment on media containing aromatic amino acids, as well as a characteristic almondlike smell on phenylalanine agar (17). Furthermore, they have the other characteristics of this genus (Table 3). They are negative for the following tests: lysine and ornithine decarboxylases, arginine dihydrolase, deoxyribonuclease, lipase (both corn oil and Tween 80), gelatinase, H2S production on Klglers and triple sugar iron agars, malonate, mucleate, acetate, tartrate, pectate, o-nitrophenyl-β-D-galactopyranoside, Voges-Proskauer, and acid production from L-arabinose, cellobiose, dulcitol, D-lactose, melibiose, a-methyl-glucoside, raffinose, and D-sorbitol. They are positive for the following tests: acid production from L-mannose, methyl red, phenylalanine deaminase, and tyrosine clearing. Providencia heimbachae can be differentiated from other Providencia species by its negative tests for Simmons citrate, growth in the presence of KCN, acid production from trehalose, urease, and indole production and by positive tests for acid production from adonitol, D-arabitol, D-galactose, and L-rhamnose (Table 5). Motility at 36°C is variable and weak (46% positive after 2 days; 85% positive after 3 to 7 days), and the gas produced in small amounts from D-glucose, D-galactose, and L-mannose by some strains is like that produced by Providencia rustigianii (often a pinhead-sized gas bubble in a Durham tube). Additional characteristics of Providencia heimbachae are given in Tables 3 through 5.

Some biochemical tests give different results when medium ingredients or methods are varied. The indole reaction is negative when bacteriological neutralized peptone (Oxoid USA). Polypeptone (BBL), or casein hydrolysate (E. Merck AG, Darmstadt, Federal Republic of Germany) is used; however, it is weakly positive after 2 to 7 days when tryptone (Oxoid USA) or peptone is used along with 0.1% tryptophan (2). The citrate reaction is negative only under standardized conditions (4). Traces of readily metabolized substances, such as peptone or glucose, may lead to false-positive results. The KCN test is negative under standardized conditions, as described previously (5); however, weak positive results are obtained if the test is performed as described by Cowan (3) or Munson (13).

The similar biochemical reactions of Providencia heimbachae and Providencia rustigianii, including weak or delayed motility, weak or no production of gas from sugars, and inability to utilize acetate, citrate, or tartrate, indicate that these two species are phenotypically more similar to each other than to the other three species of Providencia. The similarity between Providencia heimbachae and Providencia rustigianii may in part reflect that the common habitat of the two species (i.e., the intestines of healthy penguins [12] and probably also other animals).

Description of the type strain. The type (holotype) strain of Providencia heimbachae is strain MUA 2-110 (= CDC 8025-83 = ATCC 35613). This strain was isolated from the
feces of a penguin (*Aptenodytes patagonica*) in a West German zoo by F. Heimbach. Its phenotypic characteristics (Table 3) are essentially those of the species.

**LITERATURE CITED**


