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Aerococcus urinae: Intraspecies Genetic and Phenotypic Relatedness

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A number of *Aerococcus*-like organisms were recently recognized as human pathogens. Five *Aerococcus*-like strains were proposed as members of the new species *Aerococcus urinae* (with type strain E2 [= NCTC 12142]) on the basis of the results of a 16S rRNA sequence analysis. The intraspecies phenotypic and genetic relatedness of 22 selected *A. urinae* strains was investigated, and a hitherto unrecognized esculin hydrolysis-positive biotype was identified. A total of 14 of the 15 more common esculin-negative strains exhibited very high DNA relatedness as determined by the hydroxyapatite method (the levels of relatedness were greater than 90% in 55 and 70°C reactions, with 1.5% or less divergence in related sequences). The DNA relatedness among the six esculin-positive strains was more heterogeneous, and two DNA hybridization subgroups were formed. Our results are compatible with the hypothesis that both biotypes are members of the single species *A. urinae*, which contains two or more genetic subspecies. The putative subspecies have not been formally proposed since they cannot be definitively differentiated. The inclusion of *A. urinae* in the genus *Aerococcus* is supported by the results of 16S rRNA sequencing. The rRNA sequence data also is compatible with placing both biotypes in a single species.

Recently, some *Aerococcus*-like organisms were recognized and characterized as human pathogens (5–8). These bacteria were isolated from urine specimens from elderly patients suffering from urinary tract infections (5, 7) and from blood cultures taken from patients with endocarditis and urosepticemia (6, 8). The *Aerococcus*-like organisms were described by Aguirre and Collins as members of a new species of the genus *Aerococcus*, *Aerococcus urinae*, based on the results of a partial 16S ribosomal nucleic acid sequence analysis (1). These authors sequenced short fragments of 16S rRNA corresponding to *E. coli* 16S positions 50 to 450 and 950 to 1210 (1, 4) that included four variable regions from five phenotypically identical *Aerococcus*-like strains, including type strain E2 (= NCTC 12142), and found that these fragments were 100% homologous. The sequence of 1,481 nucleotides of the 16S rRNA of one representative strain (strain $E2^T$ [T = type strain]) was determined. The primary sequence of this organism was aligned and compared with the 16S rRNA sequences of 28 reference strains, including *Aerococcus viridans* strains. The *Aerococcus*-like organism sequence exhibited 94.5% similarity to the *A. viridans* sequence, 91 to 92% similarity to the sequences of carnobacteria, enterococci, and vagococci, and 86 to 90% similarity to the sequences of members of other genera, including the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus*. The *Aerococcus*-like organisms could be differentiated phenotypically from *A. viridans*. Because of these findings, Aguirre and Collins concluded that the *Aerococcus*-like organisms represent a second line within the genus *Aerococcus*, which they described as the new species *A. urinae* (1).

During the last decade strains resembling the Danish *A. urinae* strains have been received for identification at the Centers for Disease Control and Prevention (CDC). Most of these strains were able to hydrolyze esculin, in contrast to the Danish isolates. The CDC and Danish *A. urinae* strains were tested to determine intraspecies relatedness by examining phenotypic characteristics, DNA relatedness, and partial 16S ribosomal nucleic acid sequences. In this study the separation of these organisms from *A. viridans* and their relatedness to *A. viridans* were investigated in relation to the previous findings of Aguirre and Collins (1).

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MATERIALS AND METHODS

Bacterial strains. The 22 *A. urinae* strains included in the present study are listed in Table 1; 7 non-Danish strains were obtained from the culture collection of the Streptococcus Laboratory, CDC, Atlanta, Ga. In addition, the following strains were used in the DNA reassociation studies: *A. viridans* ATCC 11563^T, SS-930 (2), and 1679-93 and *Pediococcus urinaeequi* ATCC 29723^T.

Phenotypic characterization of *A. urinae* strains. Phenotypic characteristics of strains were determined by conventional tests as described by Facklam and Elliott (13).

DNA reassociation studies. The strains used for DNA extraction were grown in 2 liters of Todd-Hewitt broth at 37°C for 18 to 20 h with gentle shaking. Cells were harvested by centrifugation and were resuspended in a solution containing 50 ml of TS buffer (50 mM Tris buffer [pH 8.0] containing 12.5% sucrose), 10 ml of a lysozyme solution (20 mg/ml; Sigma Chemical Co., St. Louis, Mo.), and 1 ml of mutanolysin (2,000 U/ml; Sigma Chemical Co.). After 1 h of incubation at 37°C, 20 ml of a 50 mM EDTA solution and 0.33 ml of a proteinase K solution (25 mg/ml; Sigma Chemical Co.) were added, and the suspension was incubated for 1 h at 37°C. Lysis was completed by adding 10 ml of a 10% sodium dodecyl sulfate solution. The procedures used to purify DNA for determinations of DNA relatedness in free solution by the hydroxyapatite hybridization method have been described previously (3). The DNAs were labeled enzymatically in vitro with [^{32}P]dCTP by using a nick translation reagent kit (Gibco BRL Life Technologies, Inc., Gaithersburg, Md.) as directed by the manufacturer. DNA hybridization experiments were performed at 55°C for optimal DNA reassociation and at 70°C for stringent DNA reassociation. Levels of divergence within related sequences were determined by assuming that each 1°C of DNA heteroduplex instability, compared with the melting temperature of the homologous DNA duplex, was caused by approximately 1% unpaired bases. Levels of divergence were calculated to the nearest 0.5%.

PCR amplification of rDNA and 16S rDNA sequencing. Template DNAs for sequencing were prepared by enzymatic amplification of the 16S rRNA genes of *A. viridans* ATCC 11563^T and *A. urinae* $E2^T$, 1656-92, G1-84, F1-84, 998-93, and 944-94. Modified versions of primers fd1 and rd1 (11, 20) were used in the PCR;

TABLE 1. Strains of *A. urinae* examined in this study

Strain	Source	Sender[a]
Danish strains		
E2^T (= NCTC 12142^T = NCFB 2893^T)	Urine	Bispebjerg Hospital DCM
G1-84	Urine	Bispebjerg Hospital DCM
F1-84	Urine	Bispebjerg Hospital DCM
R7-89	Urine	Rigshospitalet DCM
R8-89	Urine	Rigshospitalet DCM
Å11-89	Urine	Ålborg Hospital DCM
BBH-B1-89	Blood	Bispebjerg Hospital DCM
FH-B1-89	Blood	Frederiksberg Hospital DCM
HER-B1-92	Blood	Herlev Hospital DCM
HER-B2-93	Blood	Herlev Hospital DCM
HIL-B1-91	Blood	Hillerod Hospital DCM
ÅLB-B1b-93	Heart valve	Ålborg Hospital DCM
ÅLB-B1a-93	Blood	Ålborg Hospital DCM
ÅRH-B2-94	Blood	Århus Hospital DCM
Non-Danish strains		
998-93	Urine	New York SHD
1656-92	Urine	New York SHD
1515-85	Urine	A. W. Sturm, The Netherlands
1871-94	Urine	New York SHD
944-94	Urine	Ohio SHD
3352-95	Urine	M. Lovgren, Edmonton, Alberta, Canada
1667-95	Urine	M. Lovgren, Edmonton, Alberta, Canada

[a] DCM, Department of Clinical Microbiology; SHD, State Health Department.

the enzyme linker region was omitted from each primer. The PCR conditions used have been described previously (21). The DNA produced was purified from the reaction mixture by filtration through a Centricon column (Amicon, Beverly, Mass.) before sequencing. The 16S ribosomal DNAs (rDNAs) were sequenced by using Amplitaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.) and dye-labeled dideoxynucleotides (Applied Biosystems, Inc., Foster City, Calif.). The primer set used for sequencing was derived from the primer sets described by Stackebrandt and Charfreitag (18). The resulting DNA fragments were separated from the cycle sequencing reaction mixture by filtration through a Centrisep column (Princeton Separations, Adelphia, N.J.). The fragments were then resolved on 6% polyacrylamide gels containing 8 M urea in an automated sequencer (model 373A; Applied Biosystems, Inc.), and data were collected and plotted on a Macintosh computer equipped with compatible software (Applied Biosystems, Inc.). The sequences were edited and analyzed by using DNASTAR software (DNASTAR, Inc., Madison, Wis.). The 16S rDNA sequences used in a multiple-sequence alignment were retrieved from GenBank and were aligned by using PILEUP from the Genetics Computer Group (12). PHYLIP version 3.51c (15) software was used to compute phylogenetic relationships based on a continuous stretch of 1,320 nucleotides of the 16S rDNA. A similarity matrix was computed by the method described by Jukes and Cantor (16). A dendrogram was constructed by the neighbor-joining method described by Saitou and Nei (17). The designations and GenBank nucleotide sequence accession numbers for strains whose 16S rRNA sequences were used to construct the dendrogram in Fig. 1 are shown in Table 2.

TABLE 2. Strain designations and GenBank accession numbers or references for 16S rRNA gene sequences used to construct the dendrogram

Organism	Strain	Accession no. or reference
Aerococcus urinae	E2^T	M77819
Aerococcus urinae	F1-84	U64456
Aerococcus urinae	G1-84	U64457
Aerococcus urinae	1656-92	U64458
Aerococcus urinae	944-94	U64459
Aerococcus urinae	998-93	U64460
Aerococcus viridans	ATCC 11563	M58797
Bacillus anthracis	Storne	X55059
Bacillus cereus	NCDO 1771	X55060
Bacillus subtilis	NCDO 1769	X60646
Brochothrix campestris	ATCC 43754	X56156
Brochothrix thermosphacta	NCDO 1676	X56155
Carnobacterium divergens	NCDO 2763	X54270
Carnobacterium gallinarum	NCFB 2766	X54269
Carnobacterium piscicola	NCDO 2762	X54268
Gemella haemolysans	ATCC 10379	L14326
Gemella morbillorum	ATCC 27824	L14327
Helcococcus kunzii	NCFB 2900	9
Lactobacillus delbreuckii	DSM 20074	M58814
Lactobacillus confusus	NCDO 5186	X52567
Lactococcus gurvieae	NCDO 2155	X54262
Lactococcus lactis	NCDO 2118	X54260
Lactococcus raffinolactis	NCDO 617	X54261
Leuconostoc mesenteroides	DSM 20343	M23035
Listeria monocytogenes	ATCC 35152	M58822
Pediococcus damnosus	NCDO 1832	10
Pediococcus parvulus	NCDO 1634	10
Pediococcus pentosaceus	DSM 20336	10
Pediococcus urinaeequi	NCDO 1636	10
Streptococcus mutans	NCTC 10499	X58303
Streptococcus parasanguis	ATCC 15912	X53652
Streptococcus pyogenes	NCDO 2381	X59029
Streptococcus sanguis	NCTC 7863	X53653
Vagococcus fluvialis	NCDO 2497	X54258
Vagococcus salmoninarum	NCFB 2777	X54272

RESULTS

All 22 *A. urinae* strains were α-hemolytic, gram-positive cocci that occurred predominantly in clumps. All of these strains gave positive reactions in the following tests: vancomycin sensitivity; production of leucine aminopeptidase; growth in the presence of 6.5% NaCl; growth at 45°C; hippurate hydrolysis; and acid production from D-mannitol, D-sorbitol, and sucrose. All of the strains gave negative reactions in the following tests: catalase; motility; pigmentation; production of pyrrolidonyl aminopeptidase; gas production from MRS broth; bile-esculin; growth at 10°C; deamination of arginine; production of urease; hydrolysis of starch; utilization of pyruvate; tellurite; litmus milk reaction; acetoin production; and acid production from L-arabinose, glycerol, inulin, lactose, melibiose, raffinose, sorbose, and trehalose. On 5% sucrose agar the strains were mucoid and nonadherent; in 5% sucrose broth they were not gelled or viscous, nor did they produce deposits. Acid was produced from maltose by four strains (one esculin-positive strain and three esculin-negative strains). Six isolates were esculin hydrolysis positive, which provided the basis for two biotypes. The esculin-negative biotype included all of the Danish strains and a single non-Danish strain, while the esculin-positive biotype contained only non-Danish isolates.

A. urinae E2^T, G1-84, 1656-92, and 998-93 were radiolabeled for use in DNA reassociation experiments (Table 3). Strains E2^T and G1-84 were very closely related; these organisms exhibited 99% relatedness at 55°C as well as at 70°C, and the level of divergence in related DNA sequences was less than 0.5%. Labeled DNAs from these two esculin-negative strains exhibited a median level of relatedness of 96% (range, 90 to 99%) to 12 other esculin-negative *A. urinae* isolates in reactions at 55°C; the levels of divergence were between 0 and 2.0%. In reactions at 70°C the median level of relatedness was 98% (range, 92 to 100%). Strain 998-93, although phenotypically identical to the labeled strains, exhibited relative binding ratios (RBR) of 66 and 61% at the optimal and stringent temperatures, respectively, and a level of divergence of 5.5%. In the reciprocal reactions, labeled DNA from strain 998-93 exhibited higher levels of relatedness to E2^T; the RBR were 83 and 69% at the optimal and stringent incubation temperatures,

TABLE 3. Intraspecies DNA relatedness of *A. urinae* strains

Source of unlabeled DNA	Source of labeled DNA[a]											
	Strain E2^T			Strain G1-84			Strain 1656-92			Strain 998-93		
	RBR at 55°C (%)	% Divergence	RBR at 70°C (%)	RBR at 55°C (%)	% Divergence	RBR at 70°C (%)	RBR at 55°C (%)	% Divergence	RBR at 70°C (%)	RBR at 55°C (%)	% Divergence	RBR at 70°C (%)
Biotype 1 strains (esculin negative)												
E2^T	100	0.0	100				63	5.0	55	83	4.5	69
G1-84	99	0.0	99	100	0.0	100	68	5.5	57			
FH-B1-89	99	0.5	99	98	1.0	99						
F1-84				98	1.5	100						
BBH-B1-89				99	2.0	100						
HER-B1-92				97	1.0	97						
HER-B2-93				96	1.5	98						
HIL-B1-91				91	1.0	95						
ÅLB-B1b-93				94	1.0	99						
ÅLB-B1a-93				96	1.0	98						
ÅRH-B2-94				90	1.0	92						
R7-89	97	0.0	99									
R8-89	93	0.0	92									
Å11-89	90	0.0	94									
998-93	66	5.5	61				79	4.0	64	100	0.0	100
Biotype 2 strains (esculin positive)												
1656-92				74	6.0	62	100	0.0	100	78	4.0	67
1515-85				81	6.0	68	73	5.5	61			
3352-95	69	5.5	60				70	5.0	57			
944-94							93	3.0	93			
1871-94							93	3.0	94			
1667-95	64	4.5	59				96	0.0	97			

[a] The values are the averages of the values from two experiments. Before normalization to 100% the levels of DNA bound to hydroxyapatite in homologous reactions were 54 to 78%. The levels of labeled DNA that bound to hydroxyapatite in control reaction mixtures that did not contain unlabeled DNA were 0.5 to 3.5% at 55°C and 0.5 to 3.0% at 70°C; these control values were subtracted from all reassociation reaction values before normalization.

respectively, and the level of divergence was 4.5%. The median levels of relatedness of strains E2^T and G1-84 to four of the esculin-positive *A. urinae* isolates were 72% (range, 64 to 81%) at 55°C and 62% (range, 59 to 68%) at 70°C, with levels of divergence between 4.5 and 6.0%. Labeled DNA from esculin-positive strain 1656-93 was highly related to DNAs from three of the other five esculin-positive strains, with RBR greater than 93% and levels of divergence of ≤3.0%, whereas for two esculin-positive strains the RBR were lower and the level of divergence was 5.5% and thus the values were comparable to the values obtained when these strains were compared to E2^T and G1-84. Thus, all of the esculin-negative strains except strain 998-93 comprised a very homogeneous group, and these strains were closely related to, but slightly genotypically different from, the esculin-positive strains. Strain 998-93 was also slightly genotypically different from esculin-negative strain E2^T and esculin-positive strain 1656-92. Strain E2^T exhibited RBR of 10 to 14% with the *A. viridans* strains and *P. urinaeequi* at 55°C.

16S rDNA sequences of *A. urinae* E2^T, 1656-92, G1-84, F1-84, 944-94, 998-93, *A. viridans* ATCC 11563^T, and *P. urinaeequi* ATCC 29723^T were determined. The *A. urinae* strains exhibited 98.2 to 99.3% similarity over 1,320 bases (Table 4). There were 7 to 10 nucleotide differences between the esculin-

TABLE 4. Levels of similarity based on a comparison of 1,320 nucleotides of the 16S rRNA genes of *A. viridans*, *A. urinae*, *P. urinaeequi*, and *Pediococcus damnosus*[a]

Taxon	% Similarity								
	A. viridans	*A. urinae* E2^T	*A. urinae* F1-84	*A. urinae* 1656-92	*A. urinae* 944-94	*A. urinae* 998-93	*A. urinae* G1-84	*P. urinaeequi*	*P. damnosus*
A. viridans	100.00								
A. urinae E2^T	93.51	100.00							
A. urinae F1-84	92.93	99.32	100.00						
A. urinae 1656-92	93.67	99.33	98.82	100.00					
A. urinae 944-94	92.77	98.30	97.76	98.98	100.00				
A. urinae 998-93	93.56	98.42	97.89	98.92	97.86	100.00			
A. urinae G1-84	92.64	98.24	97.57	97.82	96.75	98.06	100.00		
P. urinaeequi	99.56	93.54	92.94	93.72	92.77	93.88	92.98	100.00	
P. damnosus	86.49	85.79	85.30	85.79	85.22	85.93	86.04	87.64	100.00

[a] The sequence alignment was corrected for multiple base changes by the method of Jukes and Cantor (16).

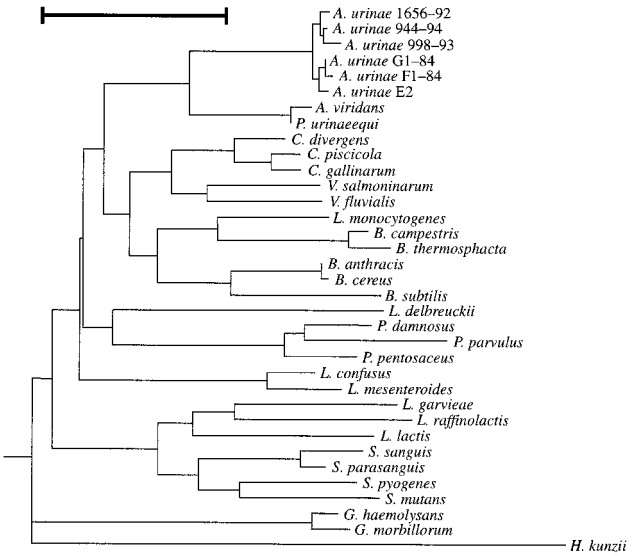

FIG. 1. Dendrogram based on an alignment of 1,320 nucleotides of the 16S rRNA genes of *A. urinae* and other gram-positive, catalase-negative species. The tree was inferred from similarity values by using the neighbor-joining method of Saitou and Nei (17). The genus names, nucleotide sequence accession numbers, and designations of the strains used in this analysis are shown in Table 2. Scale bar = 5% difference in DNA sequence.

negative and esculin-positive strains and 3 nucleotide differences between the esculin-negative strains *A. urinae* F1-84 and G1-84. There were 88 to 92 nucleotide differences between two *A. urinae* strains (1656-92 and $E2^T$) and *A. viridans*. The *A. urinae* sequences were aligned with the sequences of 29 reference strains from 12 gram-positive, catalase-negative genera and formed a distinct group (Fig. 1). When *A. urinae* strains were compared to *A. viridans*, the levels of similarity were between 92.6 and 93.5%. *A. viridans* and *A. urinae* strains were on separate lines of descent. The sequence of *P. urinaeequi* was 99.6% similar to the sequence of *A. viridans*. *P. urinaeequi* was more closely related to *A. viridans* than to other *Pediococcus* spp.

DISCUSSION

The *A. urinae* strains that were initially isolated from human infections in Denmark have been shown to constitute a very homogeneous phenotypic group of bacteria (7). However, six of seven recent isolates received at the CDC Streptococcus Laboratory resembled *A. urinae* but were esculin hydrolysis positive and thus belonged to a new biotype. The ability to produce acid from maltose differed among both the esculin-positive strains and the esculin-negative strains. This phenotypic characteristic seems to be less reliable for identification, since *A. urinae* strains originally were described as maltose positive, which could not be confirmed for strains $E2^T$, G1-84, and F1-84 in the present study. *A. urinae* strains may be separated easily from other catalase-negative, gram-positive cocci that divide in two planes by differences in major important tests for this group of bacteria (Table 5). For routine laboratory identification, the leucine aminopeptidase, pyrrolidonyl aminopeptidase, and vancomycin sensitivity tests are especially useful (14).

DNA-DNA relatedness criteria that should be considered essential for including strains in a species are a level of relatedness of 70% or greater under optimal conditions for DNA reassociation and a level of divergence of less than 5% for related sequences (19). A level of relatedness of 60% or greater under stringent DNA reassociation conditions is also recommended for including strains in a species (3). Most of the esculin-negative strains fulfilled these criteria; the only exception was strain 998-93 (Table 3), which was borderline, as were four esculin-positive strains. When the esculin-positive strain 1656-92 was labeled, three of the esculin-positive strains fulfilled the criteria, whereas two strains were borderline. There appear to be at least three closely related hybridization groups which are not phenotypically distinct. We therefore do not now propose any additional subspecies or species. When more esculin-positive strains are available for study, they may be found to constitute a separate entity (either a biotype, a subspecies, or a new species). The levels of relatedness between *A. urinae* DNA and DNAs of *A. viridans* strains and *P. urinaeequi* were 10 to 14%. There is no genetic definition of a genus, although an ideal genetic genus would be a group of phenotypically similar species that are 40 to 65% related (3).

TABLE 5. Phenotypic characteristics of catalase-negative, gram-positive cocci that divide in two planes[a]

Taxon	ESC	VAN	PYR	LAP	NACl	10°C	45°C	Hem
Aerococcus urinae biotype 1	−	S	−	+	+	−	+	α
Aerococcus urinae biotype 2	+	S	−	+	+	−	+	α
Aerococcus viridans		S	+	−	+	−	−	α
Pediococcus urinaeequi		S	−	−	+	−	−	α
Alloiococcus otitidis		S	+	+	+	−	−	n
Gemella spp.		S	+	v	−	−	−	n
Helcococcus kunzii		S	+	−	+	−	−	n
Pediococcus spp.		R	−	+	v	−	v	α
Tetragenococcus halophilus		S	−	+	+	+	−	α

[a] Abbreviations: ESC, esculin hydrolysis; VAN, vancomycin disk test; S, sensitive; R, resistant; PYR, pyrrolidonyl aminopeptidase; LAP, leucine aminopeptidase; NACl, growth in broth containing 6.5% NaCl; 10°C and 45°C, growth at 10 and 45°C, respectively; Hem, hemolytic reaction on agar containing 5% sheep blood; α, alpha-hemolytic; n, no hemolysis; v, variable.

On the basis of 16S rRNA sequence comparisons, both the esculin-negative strains examined by Aguirre and Collins (1) and the esculin-negative and esculin-positive strains examined in this study exhibited great similarity. The levels of sequence similarity between *A. urinae* and *A. viridans* are 92.6 to 94.5% (1) (Fig. 1 and Table 4). Although similar levels of sequence similarity are exhibited by species in different genera, there are many examples of species in the same genus that exhibit this level or lower levels of sequence similarity. For comparison, in the genera shown in Fig. 1, *Carnobacterium piscicola* is 96.2% similar to *Carnobacterium divergens* and 96.9% similar to *Carnobacterium gallinarum*; *Streptococcus mutans* is 93.2% similar to *Streptococcus pyogenes* and 88.9 and 89.6% similar to *Streptococcus sanguis* and *Streptococcus parasanguis*, respectively; and *Bacillus anthracis* is 99.8% similar to *Bacillus cereus* and 93.6% similar to *Bacillus subtilis*. The topology of the dendrogram, with respect to branch lengths and node positions, indicates that the *Aerococcus* branch is not much different from the *Vagococcus*, *Lactococcus*, and *Streptococcus* branches. Based on the 16S rRNA sequence analysis, it seems appropriate that *A. urinae* strains are included in the genus *Aerococcus*. There is no doubt from previous studies (10) and from the present study (Table 4 and Fig. 1) that *P. urinaeequi* belongs in the genus *Aerococcus.*

A. urinae strains are found in a constant but relatively low

percentage of urine specimens (0.8%) (5). These isolates have the pathogenic potential to cause urinary tract infections. Most patients have the classical symptoms of this disease, recurrent disease episodes occur, and the distribution (i.e., number of bacteria per milliliter, singly or in admixture with other bacteria) of *A. urinae* in urine specimens resembles the distribution observed with other established urinary tract pathogens, including *Escherichia coli* and *Enterococcus faecalis* (5, 7). *A. urinae* also has the ability to cause septicemia and endocarditis; in Denmark 20 cases of bacteremia or septicemia were documented from 1987 to 1994 (8). Nearly all of the patients from whom *A. urinae* strains are isolated (from either urinary tract specimens or blood cultures) have clinical signs of acute infection. Rarely, *A. viridans* may also cause urinary tract infections, septicemia, and endocarditis; however, in a large proportion of cases, *A. viridans* strains obtained from clinical specimens are without clinical significance.

With respect to phenotypic characteristics, DNA relatedness, 16S rRNA sequences, and clinical disease spectrum, *A. urinae* and *A. viridans* exhibit similarities but they certainly also have differences. Genotypically, *A. urinae* is somewhat heterogeneous and may contain two or more subspecies. The number of deviant strains recognized thus far is insufficient to draw firm conclusions concerning this possibility. The present taxonomic status of *A. urinae* at the species level and at the genus level seems appropriate pending further data.

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