JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1993, p. 3275–3283
0095-1137/93/123275-09$02.00/0

Vol. 31, No. 12

Roseomonas, a New Genus Associated with Bacteremia and Other Human Infections

JOHN D. RIHS,[1] DON J. BRENNER,[2] ROBERT E. WEAVER,[2] ARNOLD G. STEIGERWALT,[2] DANNIE G. HOLLIS,[2] AND VICTOR L. YU[1]*

Infectious Diseases Section, Veterans Affairs Medical Center, University Drive C, Pittsburgh, Pennsylvania 15240,[1] and Meningitis and Special Pathogens Branch, Division of Bacterial and Mycotic Diseases, Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia[2]

Received 18 March 1993/Returned for modification 12 May 1993/Accepted 10 August 1993

In the 1980s, a pink bacterium different from species of the genus *Methylobacterium* was implicated in human infection. Using biochemical tests and DNA hybridization, we examined 42 strains of pink-pigmented, gram-negative bacteria that were not members of the genus *Methylobacterium*. The isolates included 6 strains each of CDC "pink coccoid" groups I, II, III, and IV; 10 isolates from Gilardi's "unnamed taxon"; and 8 blood isolates from ill, debilitated, or immunosuppressed patients. The DNA hybridization studies supported the creation of six genomospecies encompassing the 42 strains. Reactions for esculin hydrolysis, glycerol oxidation, and D-mannose oxidation enabled separation of genomospecies 1 through 4. These tests, as well as motility, nitrate reduction, citrate utilization, and oxidation of L-arabinose, D-galactose, and D-xylose, differentiated genomospecies 5 and 6 from each other and from genomospecies 1 through 4. These organisms were susceptible in vitro to the aminoglycosides, tetracycline, and imipenem and generally susceptible to the quinolones. We propose the new genus, *Roseomonas*, for these bacteria to include three named species, *Roseomonas gilardii* sp. nov., *Roseomonas cervicalis* sp. nov., and *Roseomonas fauriae* sp. nov., and three unnamed genomospecies.

Pink-pigmented, oxidative bacteria have been isolated from blood and other clinical sources over the past 25 years (5, 6, 9, 11). Gilardi and Faur (5) described seven clinical isolates (five from blood, one from cerebrospinal fluid, and one from sputum) of pink-pigmented, oxidative gram-negative bacteria that did not fit into any described species and that differed from *Methylobacterium mesophilicum* (formerly called *Pseudomonas mesophilica*) on the basis of Gram stain morphology, growth at 42°C, growth on MacConkey agar, acetate utilization, and acid production from methanol. They designated this group of organisms an "unnamed taxon."

Wallace et al. (11) studied 156 clinical isolates of pink-pigmented, gram-negative, coccoid rods received since 1966 in the Special Bacteriology Reference Laboratory at the Centers for Disease Control and Prevention (CDC). These strains were placed into "pink coccoid" groups I through IV on the basis of oxidation of D-xylose and D-mannitol and hydrolysis of esculin. Sixty of these strains were isolated from blood. In the past 5 years, similar pink-pigmented organisms have been isolated in cases of bacteremia (6, 9, 10).

In the present study, we evaluated 42 pink-pigmented strains including those described by Gilardi and Faur (5), Stefansson et al. (10), Korvick et al. (6), and Odugbemi et al. (9) and representative strains from the CDC collection (11). On the basis of the biochemical profiles and DNA relatedness among these bacteria, we propose a new bacterial genus containing six new species and present a brief overview of infection caused by these organisms.

* Corresponding author.

MATERIALS AND METHODS

Strains. Forty-two strains of pink-pigmented, gram-negative coccobacilli that were unable to utilize methanol as a sole carbon source (to distinguish them from methylobacteria) were included in this study. These included 6 strains each of CDC pink coccoid groups I, II, III, and IV; Gilardi's unnamed taxon (10 strains); and blood isolates from ill, debilitated, or immunosuppressed patients received from J. A. Korvick (2 strains), E. D. Stefansson (5 strains), and T. Odugbemi (1 strain). These strains are listed in Table 1. Type strains of *Methylobacterium* species were obtained from the American Type Culture Collection (Rockville, Md.). The pink MDA strain was obtained from Paul H. Edelstein.

Culture and biochemical studies. Phenotypic tests were performed at the Veterans Affairs Medical Center (VAMC) and at CDC. At the VAMC, tests were done with media from Regional Media Laboratories, Inc., Lenexa, Kans., incubated at 35°C in room air, and held for 7 days. Prior to inoculation for biochemical tests, strains were streaked to buffered charcoal-yeast extract (BCYE) agar and incubated until good growth was observed. Oxidase activity was detected with a 1% aqueous solution of *N,N,N,N*-tetramethyl-*p*-phenylenediamine and dihydrochloride (Becton Dickinson). Glucose fermentation was determined by using triple sugar iron agar. Oxidation of 1% carbohydrate solutions was tested in oxidation-fermentation basal medium (Hugh-Leifson). A positive reaction was recorded if the pH indicator changed from green to yellow. One-percent methanol was prepared as described by Gilardi (4). Assimilation on acetamide slants was recorded as positive if a yellow color appeared. Phenylalanine deaminase activity was determined by flooding phenylalanine agar slants with 10% ferric chloride solution. Christensen urea agar and triple sugar iron agar were used for the detection of urease and hydrogen sulfide production, respectively. Arginine dihydrolase, ly-

TABLE 1. DNA relatedness of pink-pigmented bacteria

Genomo-species	Source of unlabeled DNA	Source of isolate	% Relatedness to labeled DNA from strain[a]: 5424		E9464		E7107		C610		E7832		F4700	
			70°C (D)[b]	85°C	70°C (D)	85°C	70°C (D)	85°C	70°C (D)	85°C	70°C (D)	85°C	70°C (D)	85°C
1	Pink-pigmented 5424	Water	100 (0.0)	100			24				19			
1	5423	Water	93 (0.0)	91			26				18			
1	4857	Unknown	92 (0.0)	87			26				13			
1	5422	Water	92 (0.5)	92			26				17			
1	4853	Unknown	90 (0.0)	92			27				16			
1	5535	Blood	87 (0.0)	90			22				18			
1	E9464	Kidney transplant			100 (0.0)	100	52		7		29		23 (1.0)	5
1	4851	Unknown	79 (4.0)	67	95 (0.5)	86	30				23			
1	Seattle 7	Blood			95 (0.5)	79	27				20			
1	F5024	Eye			90 (0.0)	85	31				15			
1	5425	Water	69 (5.0)	59	81 (1.5)	75	21				20			
1	F5429	Blood			76 (1.0)	73	34				28			
1	Seattle 4	Blood			75 (0.0)	73	23				14			
1	Seattle 3	Blood			74 (1.0)	71	32				20			
1	5534	Unknown	77 (4.5)	68	72 (1.5)	66	31				19			
1	E5109	Blood			71 (1.5)	62	35				19			
1	E4177	Unknown			71 (2.5)	55	38		10		29		20 (2.5)	4
1	Seattle 2	Blood			69 (0.5)	61	24				18			
1	F5137	Wound, hip			64 (1.0)	70	35				21			
1	Seattle 8	Blood			63 (1.0)	64	20				15			
1	E5713	Blood			60 (1.0)	67	31				17			
1	5465	Unknown	75 (5.0)	66	58 (3.5)	47	30		9		18			
1	E8962	Blood			57 (0.5)	53	29				10			
2	E7107	Cervix			14		100 (0.0)	100	9		41		33 (9.5)	16
2	E3880	Lochia					100 (0.0)	67						
2	E3930	Eye					98 (0.0)	91						
2	E3809	Eye					95 (0.0)	78						
2	4843	Unknown			29 (12.0)	16	92 (2.0	80						
2	4855	Unknown					87 (2.5)	70						
2	F5163	Genital					81 (2.5)	63						
3	C610	Hand			5		17		100 (0.0)	100	7		7 (16.0)	4
3	C5755	Blood			3		14		73 (2.5)	63	9			
3	F9190	Wound, knee			5		14		70 (3.0)	59	11			
3	E5522	Sputum			6		11		67 (4.0)	63	7			
3	C5596	Blood			3		13		64 (3.0)	63	8			
4	E7832	Ear							10		100 (0.0)	100	40 (8.5)	20
4	E8516	Wound									92 (1.5)	90	46 (9.0)	19
4	F5222	Cervix									79 (0.5)	86		
5	F4700	Bone			18		53 (9.0)		11		46		100 (0.0)	100
5	E9272	Breast			13		40		10		25		75 (3.0)	64
5	F6028A (Nigeria)	Blood											71[c] (1.0)[c]	81[c]
6	F4626	Breast incision			4		12		52 (5.0)	39	2		15 (11.5)	6

Methylobacterium extorquens	3	11	8	4	
ATCC 43645	3	7	6	5	
Methylobacterium mesophilicum					
ATCC 29983	2	9	8	3	
Methylobacterium rhodinum					
ATCC 14821	3	10	6	5	
Methylobacterium fujisawaense					
ATCC 43884	3	8	7	6	
Methylobacterium organophilum					
ATCC 27886	2	9	6	6	
Methylobacterium radiotolerans					
ATCC 27329	2	8	7	4	
Methylobacterium rhodesianum					
ATCC 43882	1	9	7	4	
Methylobacterium zatmanii					
ATCC 43883	2	3	2	7	
Pink MDA					

[a] A blank space indicates that the reaction was not done.
[b] D, divergence, calculated to the nearest 0.5%.
[c] Labeled DNA from F6028.

sine decarboxylase, and ornithine decarboxylase activity were detected by using Moeller's base overlaid with sterile mineral oil. Indole production was detected by adding Kovács reagent to tryptone broth. Gelatin liquefaction was tested in nutrient gelatin stab cultures. Tolerance to 0.03% cetrimide was tested by using cetrimide agar slants. Citrate utilization was determined by using Simmons citrate slants. Salt tolerance was tested in brain heart infusion broth. Esculin hydrolysis was tested by using esculin agar slants. Nitrate reduction was determined by using nitrate broth. Absorbance of UV light was tested in a darkened room by exposing colonies grown on BCYE agar plates to a long-wave UV lamp (365 nm) at a distance of approximately 6 in. (ca. 15 cm). Absorbance of light resulted in black coloration; absence of black coloration was considered a negative reaction. Phenotypic test methods used at CDC have been described elsewhere (3).

Antimicrobial susceptibilities. Susceptibility testing was done by using dried Neg/Urine MIC type 6 panels (MicroScan Division, Baxter Healthcare Corporation, West Sacramento, Calif.). The inoculum preparation (turbidity standard technique), rehydration, and inoculation (RENOK system) were done according to the manufacturer's instructions. The panels were incubated at 35°C in room air for 48 h and read manually by using the MicroScan microdilution viewer. Interpretive criteria of the National Committee for Clinical Laboratory Standards were used to determine antibiotic susceptibility (8).

DNA methods. Cells for DNA relatedness studies were cultivated on BCYE agar. The methods used to extract and purify DNA and the hydroxyapatite method for determining DNA relatedness have been described previously (2). Since DNAs from these strains had relatively high guanine-plus-cytosine (GC) contents, 70°C was chosen as the optimal temperature for DNA reassociation and 85°C was used to determine reassociation at a stringent criterion. DNAs were labeled enzymatically in vitro with [^{32}P]dCTP by using a nick translation reagent kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) as directed by the manufacturer. GC contents were determined spectrophotometrically by the method of Marmur and Doty (7) and were reported to the nearest 0.5 mol%.

RESULTS

DNA relatedness studies. The pink-pigmented strains formed six DNA relatedness groups, or genomospecies (Table 1). We have used "genomospecies" instead of "genospecies" (1); a genomospecies is defined by DNA relatedness (12). Genomospecies 1 contained 23 strains that formed two subgroups. Average relatedness between the subgroups was 75%, with 4.5% divergence in related sequences, in 70°C reaction mixtures and 65% in 85°C reaction mixtures. Subgroup 1, represented by strain 5424, contained six strains that exhibited 91% relatedness and no divergence in related sequences in 70°C reaction mixtures and 90% relatedness in 85°C reaction mixtures. Subgroup 2, represented by strain E9464, contained 17 strains that were 73% related, with 1% divergence, in 70°C reaction mixtures and 68% related in 85°C reaction mixtures. The closest relative of genomospecies 1 was genomospecies 2, to which it was 29% related.

Genomospecies 2, represented by strain E7107, contained seven strains that were 92% related, with 1% divergence, at 70°C and 75% related at 85°C. Its closest relatives (about 40% relatedness) were genomospecies 4 and 5.

Genomospecies 3, represented by strain C610, contained

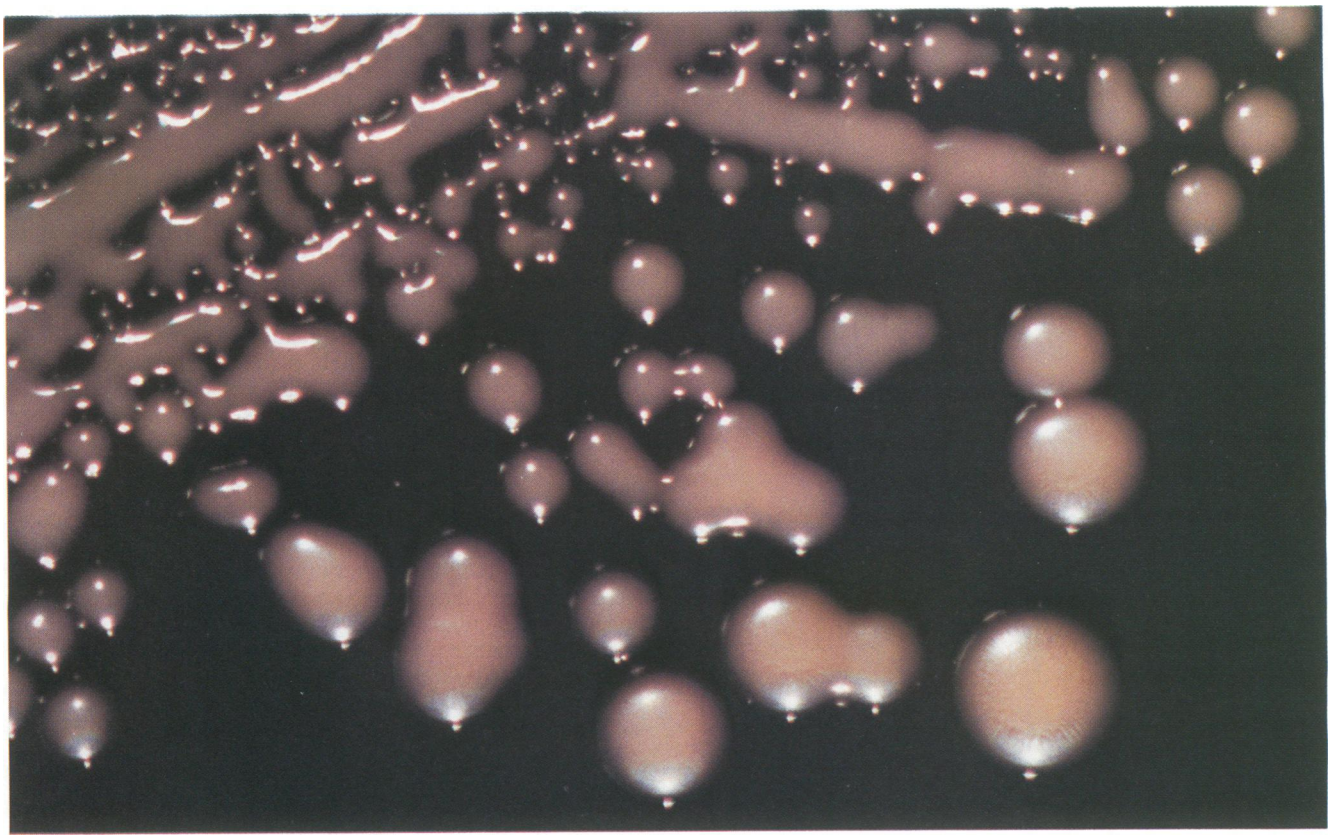


FIG. 1. Colonies of *Roseomonas* on BCYE agar.

five strains that were 69% related, with 3% divergence, at 70°C and 62% related at 85°C. The single strain of genomospecies 6, strain F4626, was the closest relative of genomospecies 3 at 52% relatedness. Labeled DNA from F4626 showed average relatedness to the five strains of genomospecies 3 of 62%, with 4.5% divergence, at 70°C and 39% at 85°C (data not shown).

Genomospecies 4 consisted of three strains, represented by strain E7832, that were 86% related, with 1% divergence, at 70°C and 88% related at 85°C. Genomospecies 4 was most closely related to genomospecies 2 and 5 (about 40%).

Genomospecies 5 consisted of three strains, represented by strain F4700, that were 73% related, with 2% divergence, at 70°C and 73% related at 85°C. Its closest relative was genomospecies 4. As stated above, the single strain F4626 was a sixth genomospecies, most closely related to genomospecies 3.

The genomospecies showed 1 to 11% relatedness to type strains of *Methylobacterium* species and to the pink MDA strain.

Culture and biochemical studies. All strains grew on 5% sheep blood agar, heart infusion agar containing 5% rabbit blood, chocolate agar, BCYE agar, and Trypticase soy agar. All strains except 2 of the 22 of genomospecies 1 strains tested grew on MacConkey agar. Colonies were pinpoint, pale pink, shiny, raised, entire, and often mucoid after 2 to 3 days of incubation at 35°C on BCYE agar (Fig. 1). Growth occurred at 25, 30, 35, and, usually, 42°C. The bacteria were weakly staining gram-negative, plump, coccoid rods, appearing in pairs or short chains, to mainly cocci with only an occasional rod.

All strains were indophenol oxidase positive (often weakly after 30 s) when tested at the VAMC, but nine strains were negative when tested at CDC. All strains were catalase positive and urease positive. Characteristics that were negative for all strains were beta-hemolysis; fermentation of D-glucose; production of indole, *o*-nitrophenyl-β-D-galactopyranoside (ONPG), and hydrogen sulfide on triple sugar iron agar; gelatin liquefaction; growth on cetrimide agar and in 6% (CDC) and 6.5% (VAMC) NaCl; deamination of phenylalanine; assimilation of acetamide; and production, by Moeller's method, of L-arginine dihydrolase, L-lysine decarboxylase, and L-ornithine decarboxylase. No strain had the ability to oxidize dulcitol, lactose, maltose, methanol, raffinose, L-rhamnose, or sucrose. No strain absorbed long-wave UV light.

Strains varied in their reactions in tests for production of oxidase (CDC), citrate utilization, esculin hydrolysis, nitrate reduction, motility, and oxidation of L-arabinose, fructose, D-galactose, D-glucose, glycerol, D-mannose, D-mannitol, salicin, and D-xylose (Table 2). Esculin, glycerol, and D-mannose could be used to differentiate genomospecies 1 through 4: genomospecies 1 was esculin negative, glycerol positive, and mannose negative with one exception; genomospecies 2 was negative in all three tests; genomospecies 3 was positive for esculin and glycerol and negative for mannose; and genomospecies 4 was negative for esculin and glycerol and positive for mannose.

Absence of motility in genomospecies 5 separated it from genomospecies 2 (Table 2). There was no single test that distinguished genomospecies 5 from genomospecies 1. However, a combination of negative glycerol oxidation and

TABLE 2. Biochemical characteristics of the pink-pigmented genomospecies

Test	No. of strains positive in genomospecies:					
	1 (n = 23)[a]	2 (n = 7)	3 (n = 5)	4 (n = 3)	5 (n = 3)	6 (n = 1)
Esculin	0	0	5	0	0	1
Glycerol	23	0	5	0	1	1
D-Mannose	1	0	0	3	0	0
L-Arabinose	15	4	5	2	1	0
Citrate	23	6	3	0	1	1
Fructose	23	7	5	2	3	1
D-Galactose	16	0	5	2	1	0
D-Glucose	9	0	1	0	0	0
MacConkey agar	20	7	5	3	3	1
D-Mannitol	13	0	0	0	0	0
Motility	8	7	5	1	0	1
Nitrate reduction	1	0	5	3	0	1
Salicin	0	1	4	0	0	1
D-Xylose	11	4	5	3	2	0

[a] n = 22 for the MacConkey agar and D-xylose tests.

negative citrate utilization in genomospecies 5 (all strains exhibited at least one of these negative characteristics) differentiated it from genomospecies 1 strains, which were positive in both of these tests. The single strain in genomospecies 6 was differentiated from genomospecies 3, with negative reactions for L-arabinose, D-galactose, and D-xylose (Table 2). Strains in all DNA relatedness groups were separable from *Methylobacterium* species by their inabilities to oxidize methanol and assimilate acetamide and by lack of absorption of long-wave UV light.

Some reactions in oxidation-fermentation medium obtained at CDC (Table 2) differed from those obtained at VAMC. All 23 strains of genomospecies 1 were negative for acid production from D-xylose at VAMC. In contrast, 11 of 22 strains examined at CDC were weakly acidic in D-xylose after incubation for 7 days. Four of the seven strains of genomospecies 2 were D-xylose positive at CDC, while only two strains were positive at VAMC. Three of the five strains of genomospecies 3 were D-glucose positive at VAMC, but only one strain was positive at CDC. The salicin reaction for E7107, one of the genomospecies 2 strains, was weakly acidic at CDC and negative at VAMC. The use of different oxidation-fermentation basal media in the two laboratories may have contributed to these differences. The medium used at VAMC is the Hugh-Leifson formulation containing the indicator bromthymol blue. The medium used at CDC (3) contains phenol red. None of these discrepant reactions affects the differentiation of the six genomospecies.

Antimicrobial susceptibilities. A summary of the 42 clinical isolates is shown in Table 3. Most of the isolates were resistant to the broad-spectrum penicillins. Seven of the 23 isolates belonging to genomospecies 1 were sensitive to carbenicillin and ticarcillin. None of the isolates in genomospecies 1 were sensitive to mezlocillin or piperacillin. None of the 15 isolates in genomospecies 2, 3, and 4 were sensitive to any of the above-mentioned penicillins. Two of the 3 isolates of genomospecies 5 were sensitive to carbenicillin and ticarcillin. The single isolate in genomospecies 6 was sensitive to ampicillin and carbenicillin only.

The penicillins containing β-lactamase inhibitors were more active in vitro. Fifteen of 23 isolates from genomospecies 1 were sensitive to amoxicillin-clavulanate, and 20 of 23 were sensitive to ticarcillin-clavulanate. All isolates from genomospecies 2 through 6 were sensitive.

TABLE 3. Antimicrobial susceptibilities of the six genomospecies

Antibiotic[a]	No. of strains sensitive in genomospecies:					
	1 (n = 23)	2 (n = 7)	3 (n = 5)	4 (n = 3)	5 (n = 3)	6 (n = 1)
Ampicillin	4	0	0	0	1	1
Carbenicillin	7	0	0	0	2	1
Mezlocillin	0	0	0	0	1	0
Ticarcillin	7	0	0	0	2	0
Piperacillin	0	0	0	0	1	0
Amox/clavulanate	15	7	5	3	3	1
Ticar/clavulanate	20	7	5	3	3	1
Cephalothin	0	0	0	2	2	0
Cefazolin	5	0	0	0	2	0
Cefoxitin	1	7	5	3	1	1
Cefotetan	2	0	5	3	3	1
Cefuroxime	0	0	1	1	0	1
Cefotaxime	1	0	3	3	3	1
Ceftriaxone	1	0	0	1	3	0
Ceftazidime	0	0	5	1	3	1
Gentamicin	23	7	5	3	3	1
Tobramycin	23	7	4	3	3	1
Amikacin	23	7	5	3	3	1
Sulfamethoxazole	0	4	4	1	3	1
Trimeth/sulfa	2	5	4	1	2	1
Tetracycline	23	7	5	3	2	1
Aztreonam	0	0	2	1	3	1
Ciprofloxacin	15	7	5	3	3	1
Norfloxacin	19	7	5	3	3	1
Imipenem	23	7	5	3	3	1
Nitrofurantoin	1	0	2	0	0	0

[a] Amox, amoxicillin; Ticar, ticarcillin; Trimeth/sulfa, trimethoprim-sulfamethoxazole.

The majority of isolates were also resistant to the cephalosporin agents. Only a few of the 23 isolates of genomospecies 1 were sensitive to any of the narrow-, expanded-, or broad-spectrum cephalosporins tested. The seven isolates of genomospecies 2 were all sensitive to cefoxitin but resistant to all other cephalosporins. The five isolates of genomospecies 3 were sensitive to cefoxitin, cefotetan, and ceftazidime. The three isolates of genomospecies 4 were sensitive to cefoxitin, cefotetan, and cefotaxime. The three isolates of genomospecies 5 were sensitive to cefotetan, cefotaxime, ceftriaxone, and ceftazidime. The isolates were generally susceptible to the quinolones. The most active antibiotics in vitro were imipenem, tetracycline, and the aminoglycosides.

DISCUSSION

Over the past 25 years, more than 170 strains of pink-pigmented bacteria have been isolated from humans (5, 6, 9–11). Almost one-half of these isolates were from blood, with about 20% from wounds, exudates, and abscesses and about 10% from genitourinary sites. Six cases in three separate reports have been presented in detail (6, 9, 10); five of these occurred in immunosuppressed or debilitated adults, and one occurred in a 9-month-old boy with epiglottitis (Table 4). In three patients from Seattle, Wash., all positive blood cultures were obtained through Hickman catheters (10). Five of the six patients recovered after antimicrobial therapy.

The most extensive study of pink-pigmented bacteria was that of Wallace et al. (11), who studied 156 clinical strains. These strains were divided into four phenotypic groups on the basis of reactions for esculin hydrolysis, oxidation of

TABLE 4. Clinical descriptions of bacteremia caused by *Roseomonas* spp.

Authors (yr)	Age of patient (yr)	Underlying disease	Infection	No. of BC positive/no. of BC drawn	Outcome
Korvick et al. (1989)	40	Acute myelogenous leukemia	Bacteremia	1/2	Recovery
	60	Intra-abdominal abscess	Bacteremia	1/2	Death
Odugbemi et al. (1988)	<1[b]	None	Epiglottitis with bacteremia	1/1	Recovery
Stefansson et al. (1988)	19	Breast cancer	Bacteremia	7/7	Recovery
	62	Crohn's disease	i.v.-associated sepsis[c]	1/2	Recovery
	57	Acute myelogenous leukemia	Bacteremia	1/1	Recovery

[a] Blood cultures (BC) in the study by Stefansson et al. were obtained via a Hickman catheter.
[b] 9 months.
[c] i.v., intravenous.

D-mannitol, and oxidation of D-xylose. Reactions for these tests were negative, negative, and positive, respectively, in phenotypic group I; all negative in phenotypic group II; negative, positive, and variable, respectively, in phenotypic group III, and positive, negative, and positive, respectively, in phenotypic group IV. All of these groups were distinguishable from the eight facultatively methylotrophic pink-pigmented species in the genus *Methylobacterium* by virtue of their inabilities to oxidize methanol and assimilate acetamide and, as shown in the present study, by their inability to absorb long-wave UV light (Fig. 2). By cellular fatty acid analysis, groups I, II, and III were not distinguishable from each other but were distinguishable from group IV (11).

In the present study, DNA hybridization studies revealed 6 genomospecies (relatedness groups) among 42 strains of these pink-pigmented bacteria. Genomospecies 1 was heterogeneous, containing two subgroups (Table 1). Subgroup 1 reference strain 5424 and five other strains in subgroup 1 were highly interrelated. Subgroup 2 contained 17 strains that were closely related on the basis of divergence within related sequences but that were heterogeneous with respect to percent relatedness in both 70 and 85°C reaction mixtures. Six of these strains exhibited less than 70% relatedness to E9464, the reference strain for subgroup 2 in 70°C reaction mixtures (although one of these, strain 5465, was 75% related to the subgroup 1 reference strain), and two of these, including strain 5465, were less than 60% related to the subgroup 2 reference strain in 85°C reaction mixtures.

Wayne et al. (12) recommended that a genetic species be defined as a group of strains that are 70% or more related (at an optimal criterion for DNA reassociation) and whose related sequences exhibit 5% or less divergence. We followed this definition, but, if strains were related at a level at or just below the borderlines of this definition, we added the criterion of 60% or more relatedness at a stringent criterion for DNA reassociation (85°C in this case). We also considered whether the borderline strains were phenotypically compatible with typical strains within the species. In genomospecies 1, all strains except E8962 fulfilled at least two of these three genetic criteria and were phenotypically indistinguishable. Strain E8962 fulfilled only one of three criteria, but it was phenotypically identical to strains of genomospecies 1. It was therefore placed, provisionally, in genomospecies 1.

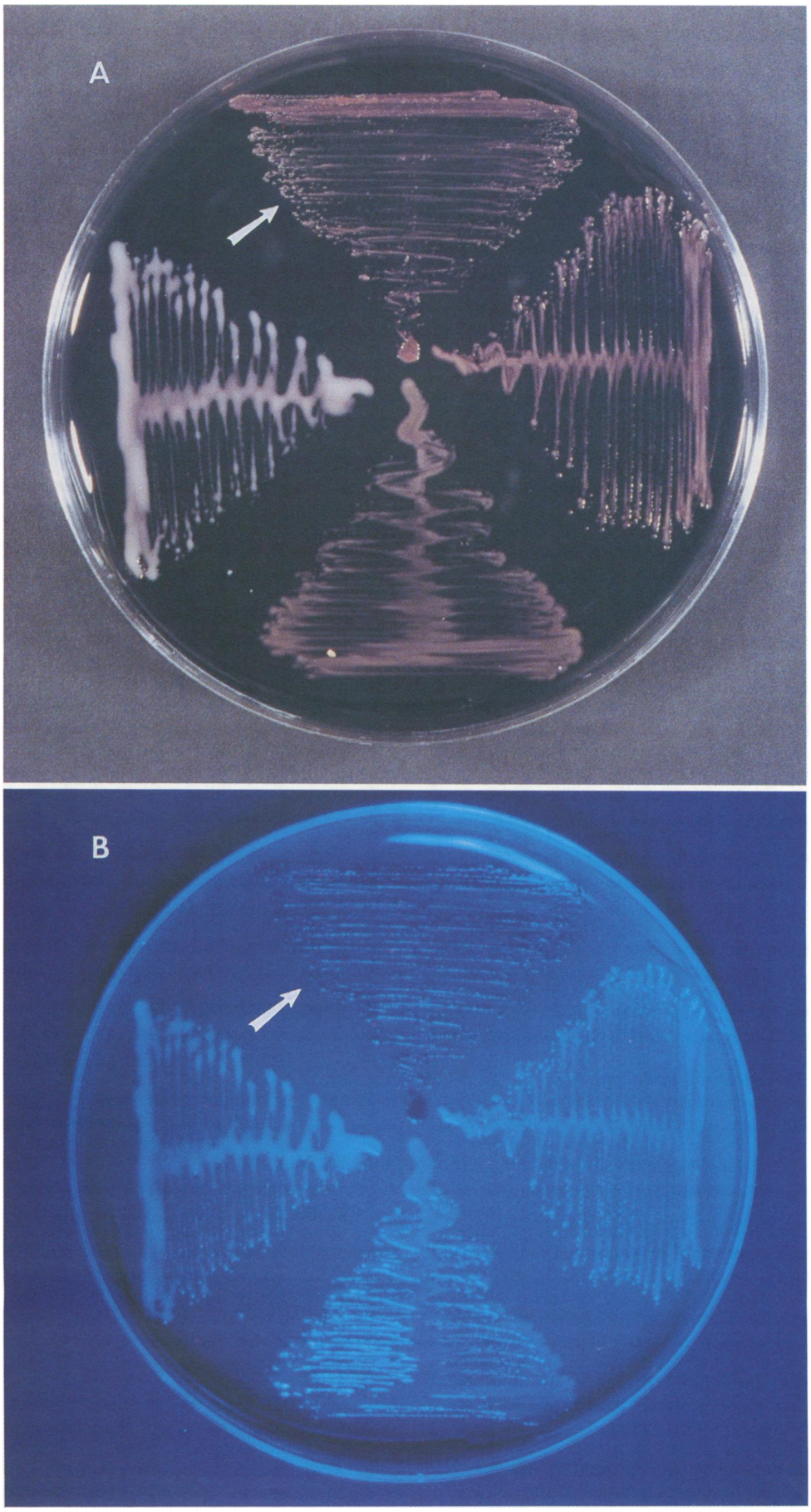
The six phenotypic group III strains of Wallace et al. used in our study corresponded to genomospecies 1. Five strains of their phenotypic group IV corresponded to genomospecies 3, and one strain, F4626, was the only member of genomospecies 6. The six strains of their phenotypic group I, however, were in genomospecies 2, 4, and 5, and the six strains from their phenotypic group II were in genomospecies 1, 2, and 5.

Reexamination of the biochemical data, in light of the genomospecies categorization, indicated that the reactions for esculin hydrolysis, glycerol oxidation, and D-mannose oxidation enabled separation of genomospecies 1 through 4 (Table 2). Lack of motility separated genomospecies 5 from genomospecies 2, and a combination of negative citrate and glycerol reactions separated genomospecies 5 from genomospecies 1. The single strain of genomospecies 6 was easily separable from genomospecies 3 on the basis of carbohydrate oxidation, as shown in Table 2.

Genomospecies 1 strains accounted for 55% of the total strains in our study and were three times as prevalent as strains in any other genomospecies. Blood isolates were most prevalent in genomospecies 1. Estimates of frequency and the rate of isolation from clinical sources remain to be defined in large-scale surveys. All strains must be characterized on the basis of esculin, glycerol, D-mannose, and other reactions (Table 2) and by DNA hybridization when necessary.

There is no genetic definition of a genus, although a general rule has been that a group should consist of species that are generally phenotypically similar and, if tested genetically, are more closely related to one another than to members of any other genus. The six genomospecies in the present study all produced a pink pigment and were quite similar biochemically. They were easily differentiable from other pink-pigmented bacteria, including *Methylobacterium* spp., which appeared to be their closest phenotypic and genotypic relative. Genetic relatedness between genomospecies was higher than the relatedness of any genomospecies to *Methylobacterium* spp. Genomospecies 3, however, exhibited only 7 to 17% relatedness to other genomospecies and 6

FIG. 2. Differentiation of three *Roseomonas* bacteria from one *Methylobacterium* sp. using long-wave UV light. (A) Incandescent light. Various shades of pink are seen for all bacteria. (B) UV light. *Methylobacterium* sp. (top arrow) appears dark because of its absorption of UV light.


A
B

to 8% relatedness to *Methylobacterium* spp. Despite its low level of DNA relatedness, genomospecies 3, for the present, is best placed in the same genus as the other genomospecies. This is due to its overall phenotypic similarity to other genomospecies and the lack of a good reason to create a separate genus for it. When one must choose between a genomic and a phenotypic basis for determining a genus, it is best to choose on the basis of phenotype (12). We thus propose a single new genus for these bacteria. This proposal and proposals for three named species and three unnamed genomospecies are given below.

Description of the genus *Roseomonas* gen. nov. *Roseomonas* (Roz.e.o.mo'nas M.L. adj. *roseus*, rosy, rose-colored, or pink; Gr. n. *monas*, a unit; M.L. n. *Roseomonas*, a pink-pigmented bacterium). Members of the genus are gram-negative, nonfermentative, plump coccoid rods, appearing in pairs or short chains, to mainly cocci with only an occasional rod. They grow on 5% sheep blood agar, heart infusion agar with 5% rabbit blood, chocolate agar, BCYE agar, Trypticase soy agar, and almost always (91%) on MacConkey agar but do not grow in media containing 6% or more NaCl. Growth occurs at 25, 30, 35, and, usually, 42°C. Pinpoint, pale-pink, shiny, raised, entire, and often mucoid colonies appear on BCYE agar after 2 to 3 days of incubation at 35°C. They are catalase and urease positive. *Roseomonas* species are negative in reactions for beta-hemolysis, indole production, ONPG, hydrogen sulfide production, gelatin liquefaction, phenylalanine deaminase, acetamide assimilation, growth in the presence of cetrimide, L-lysine and L-ornithine decarboxylases, and L-arginine dihydrolase. They do not oxidize dulcitol, lactose, maltose, methanol, raffinose, L-rhamnose, or sucrose. They do not absorb long-wave UV light. The type strains of genomospecies 1 through 4 and 6 are motile and have a single polar flagellum. The oxidase test must be considered variable, since many strains were weakly positive in tests at the VAMC and about 20% of strains were oxidase-negative in tests at CDC. Variable reactions are obtained in tests for citrate utilization, esculin hydrolysis, nitrate reduction, and oxidation of L-arabinose, fructose, D-galactose, D-glucose, glycerol, D-mannose, D-mannitol, salicin, and D-xylose. The GC content of DNA is 65 to 71 mol%. The interrelatedness of species by DNA hybridization is 7 to 53%. *Roseomonas* spp. are pathogenic for humans, causing bacteremia and wound, urinary tract, and other infections. The type species is *Roseomonas gilardii*.

Description of *Roseomonas gilardii* sp. nov. *R. gilardii* (gi.lar'di.i. M.L. gen. n. *gilardii*, to honor Gerald L. Gilardi for his many contributions to bacteriology and, specifically, for his contributions in the area of glucose-nonfermenting gram-negative rods). *R. gilardii* exhibits all of the characteristics of the genus. It corresponds to *Roseomonas* genomospecies 1. It does not hydrolyze esculin or oxidize salicin. Citrate is utilized, and fructose and glycerol are oxidized. Motility, nitrate reduction, and oxidation of L-arabinose, D-galactose, D-glucose, D-mannose, D-mannitol, and D-xylose are variable. The biochemical characteristics of use in differentiating *R. gilardii* from other *Roseomonas* species are given in Table 2. The GC contents of five strains are 67.6 to 71.2 mol%. *R. gilardii* is pathogenic for humans, causing bacteremia and other infections. The type strain is 5424 (ATCC 49956). It has a GC content of 67.6 mol%. It was isolated around 1980 from potable water.

Description of *Roseomonas cervicalis* sp. nov. *R. cervicalis* (cer.vi.ca'lis. M.L. adj. *cervicalis*, from the cervix). *R. cervicalis* exhibits all of the characteristics of the genus. It corresponds to *Roseomonas* genomospecies 2. It does not hydrolyze esculin or reduce nitrates. It is motile and oxidizes fructose but does not oxidize D-galactose, glycerol, D-glucose, D-mannitol, or D-mannose. Oxidation of L-arabinose, D-xylose, and salicin and citrate utilization are variable. The biochemical characteristics of use in differentiating *R. cervicalis* from other *Roseomonas* species are given in Table 2. The GC contents of three strains are 67.5 to 70.4 mol%. *R. cervicalis* is pathogenic for humans, causing urogenital, eye, and other infections. The type strain is E7107 (ATCC 49957). It has a GC content of 70.4 mol%. It was isolated in 1980 from the cervix of a woman in New Jersey.

Description of *Roseomonas fauriae* sp. nov. *R. fauriae* (faur'i.a.e. M.L. gen. n. *fauriae*, to honor Yvonne Faur for her contributions to public health bacteriology and, specifically, for her contribution to the recognition of pink-pigmented bacteria [5]). *R. fauriae* exhibits all of the characteristics of the genus. It corresponds to *Roseomonas* genomospecies 3. It is motile, hydrolyzes esculin, reduces nitrates to nitrites, and oxidizes L-arabinose, D-galactose, fructose, glycerol, and D-xylose but not D-mannitol or D-mannose. Utilization of citrate and oxidation of D-glucose and salicin are variable. The biochemical characteristics of use in differentiating *R. fauriae* from other *Roseomonas* species are given in Table 2. *R. fauriae* is pathogenic for humans, causing bacteremia and wound and other human infections. The type strain is C610 (ATCC 49958). It has a GC content of 68 mol%. It was isolated in 1971 from the hand wound of a woman in Hawaii.

Description of *Roseomonas* genomospecies 4. *Roseomonas* genomospecies 4 exhibits all of the characteristics of the genus. It does not hydrolyze esculin. It reduces nitrates to nitrites; oxidizes D-mannose and D-xylose; and does not utilize citrate or oxidize D-glucose, glycerol, D-mannitol, or salicin. Motility and oxidation of L-arabinose, fructose, and D-galactose are variable. The biochemical characteristics of use in differentiating *Roseomonas* genomospecies 4 from other *Roseomonas* species are given in Table 2. *Roseomonas* genomospecies 4 is presumptively pathogenic for humans, having been isolated from a wound, ear, and cervix. The type strain is E7832 (ATCC 49959). It has a GC content of 67.8 mol%. It was isolated in 1980 from the ear of a human in New York.

Description of *Roseomonas* genomospecies 5. *Roseomonas* genomospecies 5 exhibits all of the characteristics of the genus. It is nonmotile, does not hydrolyze esculin, and does not reduce nitrates. It oxidizes fructose but not D-glucose, D-mannitol, D-mannose, or salicin. Utilization of citrate and oxidation of L-arabinose, D-galactose, glycerol, and D-xylose are variable. The biochemical characteristics of use in differentiating *Roseomonas* genomospecies 5 from other *Roseomonas* species are given in Table 2.

Roseomonas genomospecies 5 is presumptively pathogenic for humans, having been isolated from blood, bone tissue, and breast tissue. The type strain is F4700 (ATCC 49960). It has a GC content of 65.0 mol%. It was isolated in 1983 from bone tissue of a male in Belgium.

Description of *Roseomonas* genomospecies 6. *Roseomonas* genomospecies 6 exhibits all the characteristics of the genus. It is motile; hydrolyzes esculin; reduces nitrates to nitrites; utilizes citrate; and oxidizes fructose, glycerol, and salicin but not L-arabinose, D-galactose, D-glucose, D-mannitol, D-mannose, or D-xylose. The biochemical characteristics of use in differentiating *Roseomonas* genomospecies 6 from other *Roseomonas* species are given in Table 2. *Roseomonas* genomospecies 6 is presumptively pathogenic for hu-

mans. The type and currently the only strain is F4626 (ATCC 49961). It has a GC content of 65.4 mol%. It was isolated in 1983 from a breast incision in a woman in Louisiana.

We propose a new bacterial genus with six species for pink-pigmented, oxidative bacteria. They are easily differentiable from *Methylobacterium* species, which appear to be their closest phenotypic and genotypic relatives. They have been increasingly isolated in the past few years from clinical specimens, especially in immunosuppressed patients. The organisms are susceptible in vitro to the aminoglycosides, tetracycline, and imipenem (Table 4). Characterization of these bacteria will facilitate identification by clinical microbiologists and likely lead to increased recognition of their pathogenicity in immunosuppressed hosts.

ACKNOWLEDGMENTS

We thank Eric D. Stefansson for allowing us to study the Seattle isolates and Shirley Brinker and Linda Szalla for manuscript preparation.

REFERENCES

1. **Brenner, D. J., P. A. D. Grimont, A. G. Steigerwalt, G. R. Fanning, E. Ageron, and C. F. Riddle.** 1993. Classification of Citrobacteria by DNA hybridization: designation of *Citrobacter farmeri* sp. nov., *Citrobacter youngae* sp. nov., *Citrobacter braakii* sp. nov., *Citrobacter werkmanii* sp. nov., *Citrobacter sedlakii* sp. nov., and three unnamed *Citrobacter* genomospecies. Int. J. Syst. Bacteriol. **43**:645–658.
2. **Brenner, D. J., A. C. McWhorter, J. K. Leete Knutson, and A. G. Steigerwalt.** 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. J. Clin. Microbiol. **15**:1133–1140.
3. **Clark, W. A., D. G. Hollis, R. E. Weaver, and P. Riley.** 1984. Identification of unusual pathogenic gram-negative aerobic and facultatively anaerobic bacteria. Centers for Disease Control, Atlanta.
4. **Gilardi, G. L.** 1978. Identification of *Pseudomonas* and related bacteria, p. 14–44. *In* G. L. Gilardi (ed.), Glucose nonfermenting gram negative bacteria in clinical microbiology. CRC Press, Inc., Boca Raton, Fla.
5. **Gilardi, G. L., and Y. C. Faur.** 1984. *Pseudomonas mesophilica* and an unnamed taxon, clinical isolates of pink-pigmented oxidative bacteria. J. Clin. Microbiol. **20**:626–629.
6. **Korvick, J. A., J. D. Rihs, G. L. Gilardi, and V. L. Yu.** 1989. A pink-pigmented, oxidative, nonmotile bacterium as a cause of opportunistic infections. Arch. Intern. Med. **149**:1449–1451.
7. **Marmur, J., and P. Doty.** 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109–118.
8. **National Committee for Clinical Laboratory Standards.** 1991. Performance standards for antimicrobial susceptibility testing; third information supplement. M100-S3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
9. **Odugbemi, T., C. Nwofor, and K. T. Joiner.** 1988. Isolation of an unidentified pink-pigmented bacterium in a clinical specimen. J. Clin. Microbiol. **26**:1072–1073.
10. **Stefansson, E. D., M. B. Agy, and L. A. McGonagle.** 1988. Gram-negative organisms that form pink, mucoid colonies cultured from the blood of three chronically ill patients. Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., Los Angeles, abstr. 667.
11. **Wallace, P. L., D. G. Hollis, R. E. Weaver, and C. W. Moss.** 1990. Biochemical and chemical characterization of pink-pigmented oxidative bacteria. J. Clin. Microbiol. **28**:689–693.
12. **Wayne, L. J., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevksy, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr, and H. G. Truper.** 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int. J. Syst. Bacteriol. **37**:463–464.