Molecular Characterization of the Genera *Proteus*, *Morganella*, and *Providencia* by Ribotyping

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The so-called *Proteus-Providencia* group is constituted at present by three genera and 10 species. Several of the recognized species are common opportunistic pathogens for humans and animals. Different methods based on the study of phenotypic characters have been used in the past with variable levels of efficiency for typing some species for epidemiological purposes. We have determined the rRNA gene restriction patterns (ribotypes) for the type strains of the 10 different species of the genera *Proteus*, *Morganella*, and *Providencia*. Visual inspection of *Eco*RV- and *Hinc*II-digested DNA from the type strains showed remarkably different patterns for both enzymes, but *Eco*RV provided better differentiation. Both endonucleases were retained to study a large number of wild and collection strains belonging to the different species. Clinical isolates of *Proteus mirabilis*, *Proteus penneri*, *Morganella morganii*, and *Providencia heimbachae* showed patterns identical or very similar to those of the respective type strains, so that groups of related patterns (ribogroups) were found to correspond to the diverse species. On the contrary, distinct ribogroups were detected within *Providencia alcalifaciens* (two ribogroups with both enzymes), *Providencia rettgeri* (four ribogroups with *Eco*RV and five with *Hinc*II), *Providencia stuartii* (two ribogroups with *Eco*RV), *Providencia rustigianii* (two ribogroups with *Hinc*II), and *Proteus vulgaris* (two ribogroups with both enzymes). The pattern shown by the ancient *P. vulgaris* type strain NCTC 4175 differed considerably from both *P. vulgaris* ribogroups as well as from the newly proposed type strain ATCC 29905 and from any other strain in this study, thus confirming its atypical nature. Minor differences were frequently observed among patterns of strains belonging to the same ribogroup. These differences were assumed to define ribotypes within each ribogroup. No correlation was observed between ribogroups or ribotypes and biogroups of *P. vulgaris*, *P. alcalifaciens*, *P. stuartii*, and *P. rettgeri*. Since, not only different species showed different rRNA gene restriction patterns, but also different ribogroups and ribotypes have been found in the majority of the species, ribotyping would be a sensitive method for molecular characterization of clinical isolates belonging to the genera *Proteus*, *Morganella*, and *Providencia*.

The classification of the *Proteus-Providencia* group in the family *Enterobacteriaceae* has been based in the past mainly on biochemical characters. Depending on the number of reactions taken into consideration by different authors at different times and the various criteria adopted for classification, the separation of the genera and species has long been questioned and submitted to periodic modifications (21). The genus *Proteus*, first described by Hauser in 1885, was successively separated into the two species *P. vulgaris* and *P. mirabilis* on the basis of ability to ferment maltose. Two further species, *P. rettgeri* and *P. morganii*, were added when other urease-producing bacteria were included in the genus *Proteus*. The DNA relatedness studies by Brenner et al. (4) not only resolved the taxonomic position of these two species but also allowed classification without controversy of the other bacteria belonging to the group. As a result, *Proteus rettgeri* was transferred to the genus *Providencia* and *Proteus morganii* was transferred to the genus *Morganella* as the sole species in the genus. At present (21), the genus *Proteus* is constituted by the four species *P. mirabilis*, *P. vulgaris*, *P. penneri*, and *P. myxofaciens*, while the genus *Providencia*, named after Providence, Rhode Island, where these enterobacteria were first studied (26), includes five species, *P. alcalifaciens*, *P. stuartii*, *P. rettgeri*, *P. rustigianii*, and *P. heimbachae*.

Several of the recognized species are common opportunistic pathogens for humans and animals, to whom they may cause primary and secondary infections. Urinary tract diseases are the most frequently observed infections. Predisposing conditions in hospital patients are catheterization and surgery of the urinary tract, while predisposing factors in outpatients are diabetes and structural abnormalities of the urinary tract. *P. mirabilis* is much more frequently responsible for infections than other components of the *Proteus-Providencia* group (21).

Serotyping, bacteriocin and bacteriophage typing, numerical analysis of electrophoretic protein patterns, plasmids, and antibiotic resistance pattern determination have been used for typing some species and have been applied to epidemiological investigations with variable efficiency (2, 7, 12, 17, 20, 25). In more recent years, molecular typing by ribosomal DNA (rDNA) fingerprints (ribotyping), based on restriction fragment length polymorphism in the chromosomal DNA containing rRNA genes (9, 18), has been applied for the epidemiological study of *P. stuartii* clinical isolates (19). The same method was compared with the arbitrarily primed PCR technique for the epidemiological investigation of an outbreak of *P. mirabilis* colonization in a pediatric hospital (3). In our study, we have determined the rRNA gene restriction patterns of collection strains and clinical isolates of the different species of the genera *Proteus*, *Providencia*, and *Morganella*, with the aim of characterizing the species of the three genera on the basis of their patterns.

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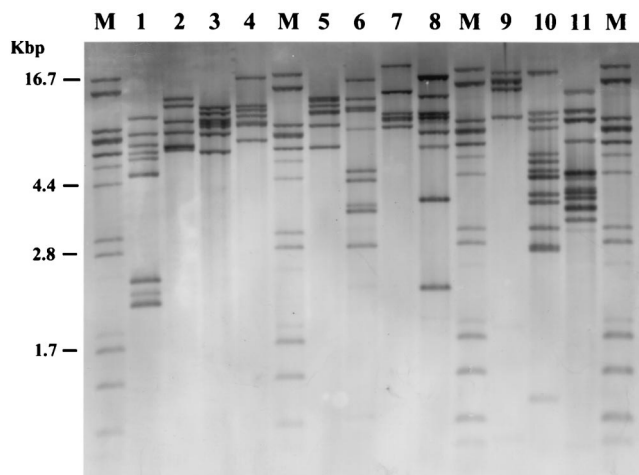
FIG. 1. *Eco*RV restriction patterns of type strains. Lanes: M, molecular weight standard; 1, *P. mirabilis* NCTC 11938; 2, *P. vulgaris* NCTC 4175; 3, *P. vulgaris* ATCC 29905; 4, *P. penneri* NCTC 12737; 5, *P. myxofaciens* NCIMB 13273; 6, *M. morganii* NCTC 235; 7, *P. alcalifaciens* NCTC 10286; 8, *P. stuartii* NCTC 11800; 9, *P. rettgeri* NCTC 11801; 10, *P. rustigianii* NCTC 11802; 11, *P. heimbachae* NCTC 12003.

FIG. 3. *Hinc*II restriction patterns of type strains. Lanes: M, molecular weight standard; 1, *P. mirabilis* NCTC 11938; 2, *P. vulgaris* NCTC 4175; 3, *P. vulgaris* ATCC 29905; 4, *P. penneri* NCTC 12737; 5, *P. myxofaciens* NCIMB 13273; 6, *M. morganii* NCTC 235; 7, *P. alcalifaciens* NCTC 10286; 8, *P. stuartii* NCTC 11800; 9, *P. rettgeri* NCTC 11801; 10, *P. rustigianii* NCTC 11802; 11, *P. heimbachae* NCTC 12003.
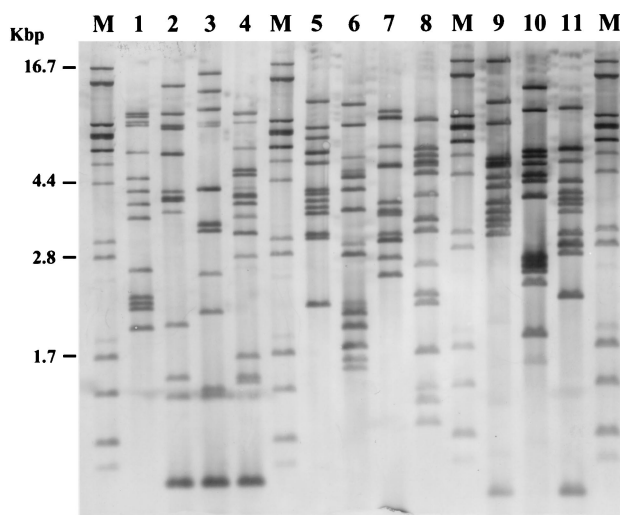
MATERIALS AND METHODS

Bacterial strains and growth conditions. A total of 78 strains belonging to the different species of *Proteus*, *Providencia*, and *Morganella* were studied. Reference strains were obtained from the National Collection of Type Cultures (NCTC), London, United Kingdom; from the National Collections of Industrial and Marine Bacteria (NCIMB), Aberdeen, Scotland; and from the Collection of the Institut Pasteur (CIP), Paris, France. Other collection strains and all clinical isolates were from the collection of the Unité des Entérobactéries, Institut Pasteur (SEIP), Paris, France; from the Centers for Disease Control and Prevention (CDC), Atlanta, Ga.; and from the Department of Hygiene and Microbiology, University of Palermo, Palermo, Italy. Most clinical strains were from urine specimens. They were randomly selected from the available isolates from the period 1990 to 1994, and their identification was based on standard biochemical methods. To further characterize our strains, biogroups were determined for strains belonging to *P. vulgaris*, *P. alcalifaciens*, *P. stuartii*, and *P. rettgeri* (14, 21, 23). Fermentation assays for biotyping were made with esculin iron agar (Difco Laboratories, Detroit, Mich.) and phenol red broth base (Merck, Darmstadt, Germany) supplemented with adonitol, inositol, rhamnose, and salicin. To obtain genomic DNA, all strains were cultured in Trypto casein soy broth (Diagnostics Pasteur, Marnes-la-Coquette, France) at 10 ml per tube, at 37°C, with the exception of *P. heimbachae* strains that were incubated at 30°C.

DNA extraction, restriction, and transfer to nylon membranes. Genomic DNA was extracted and purified by the technique of Grimont and Grimont (10). To extract genomic DNA, 2 ml of bacterial culture grown to stationary phase (18 h) was centrifuged at $10,000 \times g$ for 30 min and resuspended in 600 μl of TES buffer (50 mM Tris-HCl, 50 mM EDTA, 100 mM NaCl [pH 8]). Sodium dodecyl

sulfate (25% [wt/vol]) and pronase solutions (Calbiochem, La Jolla, Calif.) were added to give final concentrations of 1% (wt/vol) and 50 μg/ml, respectively. Lysis was achieved through a 1-h incubation at 60°C. To purify DNA, an Autogen 540 automated DNA extraction system (AutoGen Instruments, Beverly, Mass.) was used. Purified bacterial DNA (5 μg in 10 mM Tris–1 mM EDTA) was submitted to a 4-h digestion at 37°C with the buffer recommended by the manufacturer of the endonucleases (Amersham International, Amersham, United Kingdom). Digested DNA was electrophoresed at 50 V for 16 h in a horizontal 0.8% (wt/vol) agarose gel in TBE buffer (89 mM Tris base, 89 mM boric acid, 2.5 mM EDTA [pH 8]). Alkaline denaturation, neutralization, and transfer of DNA onto a nylon filter (Hybond N; Amersham) with a VacuGene apparatus (Pharmacia LKB Biotechnology, Uppsala, Sweden) were performed as previously described (16). *Mlu*I-digested genomic DNA of *Citrobacter koseri* CIP 105177 was used as a reference to determine the sizes of DNA fragments.

Hybridization with DIG-oligonucleotide probes. Hybridization with digoxigenin (DIG)-labeled rRNA OligoMix5 probes (22) and immunoenzymatic detection of hybridizing fragments were performed by using the DIG Easy Hyb kit (Boehringer, Mannheim, Germany) and the DIG nucleic acid detection kit (Boehringer) according to the recommendations provided by the manufacturer.

Determination of molecular size of fragments and computer interpretation of ribotyping banding patterns. To determine the molecular size of the fragments, banding patterns were scanned with One-Scanner (Apple Computer, Cupertino, Calif.) and then interpreted by using four programs of the Taxotron package (Institut Pasteur, Paris, France). Notably, the program RestrictoScan was used
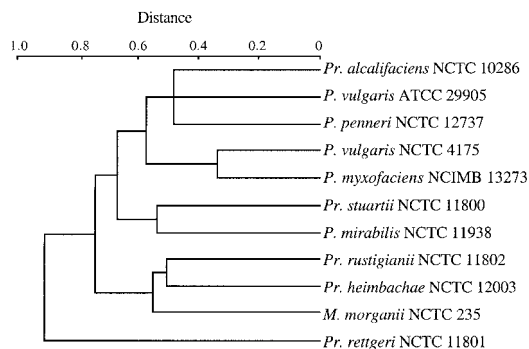
FIG. 2. Dendrogram obtained by comparison of *Eco*RV ribotyping patterns of the type strains of the 10 species studied. The distance matrix was generated by using the complement of Dice similarity coefficients and the average-linkage algorithm with a 4% tolerated error.

FIG. 4. Dendrogram obtained by comparison of *Hinc*II ribotyping patterns of the type strains of the 10 species studied. The distance matrix was generated by using the complement of Dice similarity coefficients and the average-linkage algorithm with a 4% tolerated error.

FIG. 5. Dendrogram based on the *Eco*RV ribotyping patterns of the strains listed in Table 1. The distance matrix was generated by using the complement of Dice similarity coefficients and the average-linkage algorithm with a 4% tolerated error.
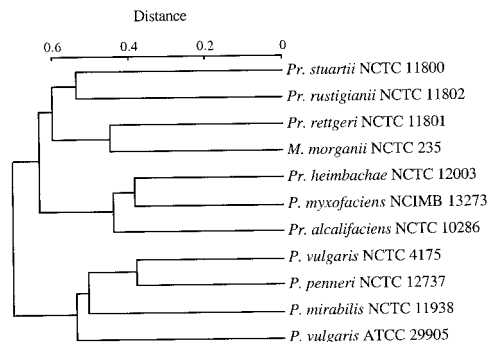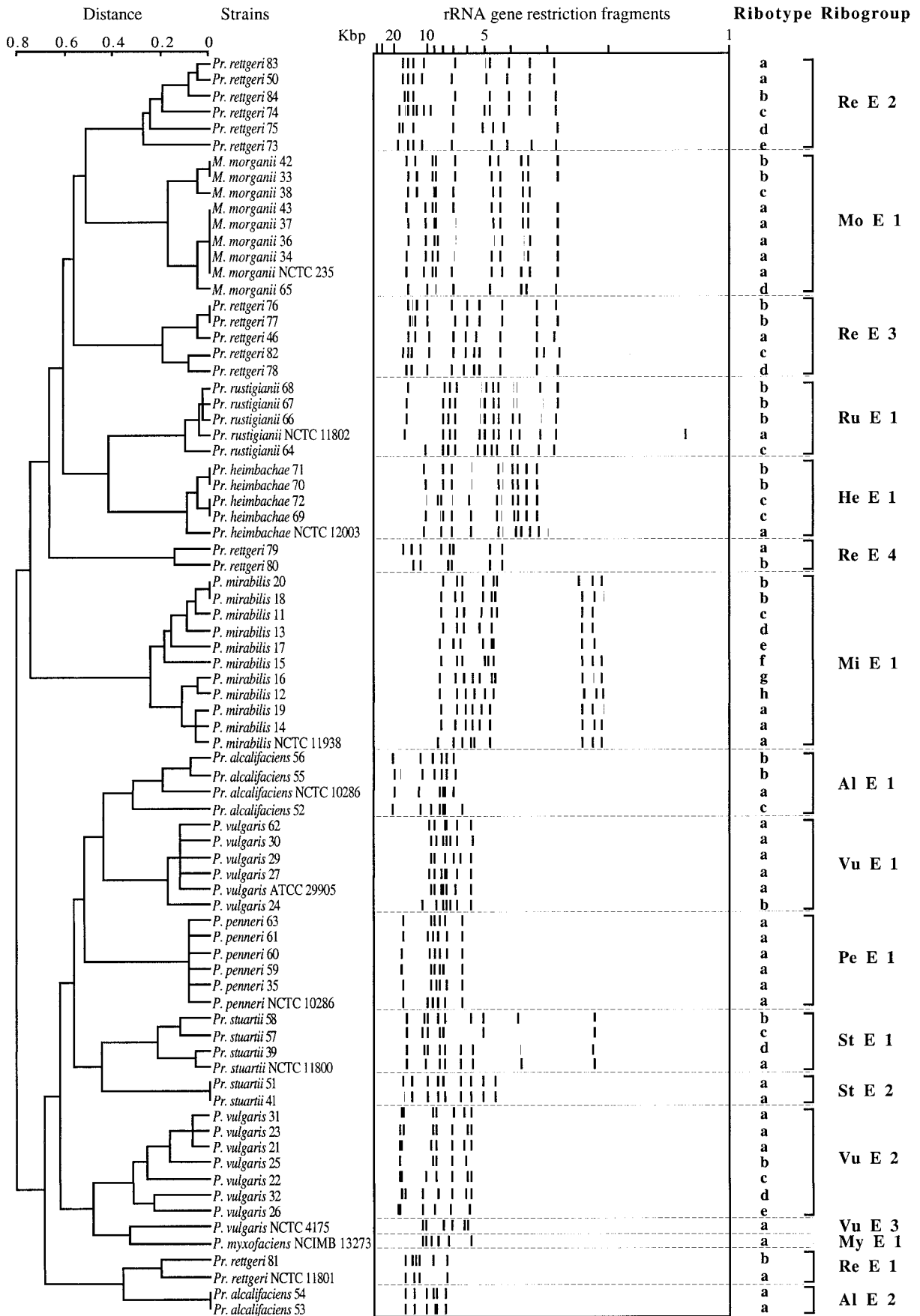
TABLE 1. Ribogroups and ribotypes of the different species of the genera *Proteus, Morganella*, and *Providencia*

Species	Strain no.[a]	Origin (yr)	Biogroup	Ribogroup or ribotype	
				*Eco*RV	*Hinc*II
P. mirabilis	NCTC 11938	Type strain		Mi E 1a	Mi H 1b
	14	Palermo, Italy (1990)		Mi E 1a	Mi H 1b
	19	Palermo, Italy (1994)		Mi E 1a	Mi H 1a
	18	Palermo, Italy (1992)		Mi E 1b	Mi H 1c
	20	Palermo, Italy (1994)		Mi E 1b	Mi H 1f
	11	Palermo, Italy (1990)		Mi E 1c	Mi H 1e
	13	Palermo, Italy (1990)		Mi E 1d	Mi H 1d
	17	Palermo, Italy (1992)		Mi E 1e	Mi H 1g
	15	Palermo, Italy (1992)		Mi E 1f	Mi H 1a
	16	Palermo, Italy (1992)		Mi E 1g	Mi H 1c
	12	Palermo, Italy (1990)		Mi E 1h	ND[b]
P. vulgaris	ATCC 29905	New type strain	2	Vu E 1a	Vu H 1a
	62 (1.83)	Paris, France (1983)	Atypical	Vu E 1a	Vu H 1e
	27	Palermo, Italy (1992)	2	Vu E 1a	Vu H 1c
	30	Palermo, Italy (1992)	2	Vu E 1a	ND
	29	Palermo, Italy (1992)	3	Vu E 1a	Vu H 1b
	24	Palermo, Italy (1991)	2	Vu E 1b	Vu H 1d
	21	Palermo, Italy (1990)	2	Vu E 2a	Vu H 2b
	23	Palermo, Italy (1991)	2	Vu E 2a	Vu H 2d
	31	Palermo, Italy (1993)	2	Vu E 2a	Vu H 2g
	25	Palermo, Italy (1991)	3	Vu E 2b	Vu H 2c
	22	Palermo, Italy (1990)	2	Vu E 2c	Vu H 2e
	32	Palermo, Italy (1993)	3	Vu E 2d	Vu H 2f
	26	Palermo, Italy (1991)	3	Vu E 2e	Vu H 2a
	NCTC 4175	Former type strain	3	Vu E 3a	Vu H 3a
P. penneri	NCTC 12737	Type strain		Pe E 1a	Pe H 1a
	35	Palermo, Italy (1991)		Pe E 1a	Pe H 1b
	59	ATCC 33519		Pe E 1a	ND
	60 (6.88)	Paris, France (1988)		Pe E 1a	ND
	61 (8.88)	Paris, France (1988)		Pe E 1a	ND
	63 (9.89)	Nantes, France (1989)		Pe E 1a	ND
P. myxofaciens	NCIMB 13273	Type strain		My E 1a	My H 1a
M. morganii	NCTC 00235	Type strain		Mo E 1a	Mo H 1a
	33	Palermo, Italy (1990)		Mo E 1b	Mo H 1c
	34	Palermo, Italy (1990)		Mo E 1a	Mo H 1f
	36	Palermo, Italy (1991)		Mo E 1a	Mo H 1b
	37	Palermo, Italy (1992)		Mo E 1a	Mo H 1g
	43	Palermo, Italy (1991)		Mo E 1a	ND
	42	Palermo, Italy (1990)		Mo E 1b	ND
	38	Palermo, Italy (1993)		Mo E 1c	Mo H 1e
	65	CDC 1143.76		Mo E 1d	Mo H 1d
P. alcalifaciens	NCTC 10286	Type strain	1	A1 E 1a	A1 H 1a
	55 (4.75)	Toulon, France (1975)	1	A1 E 1b	A1 H 1c
	56 (3.79)	Rives, France (1979)	1	A1 E 1b	A1 H 1b
	52	CDC 3370.67	1	A1 E 1c	A1 H 1d
	53 (1.84)	Montigny, France (1984)	1	A1 E 2a	A1 H 2a
	54 (10.80)	Paris, France (1980)	1	A1 E 2a	A1 H 2a
P. stuartii	NCTC 11800	Type strain	5	St E 1a	St H 1a
	58 (3.85)	Nigeria (1985)	5	St E 1b	ND
	57 (3.89)	Briançon, France (1989)	5	St E 1c	ND
	39	Palermo, Italy (1991)	5	St E 1d	ND
	41	Palermo, Italy (1994)	5	St E 2a	St H 1c
	51	Palermo, Italy (1993)	5	St E 2a	St H 1b
P. rettgeri	NCTC 11801	Type strain	2	Re E 1a	Re H 1a
	81 (2.71)	Tangier, Morocco (1971)	2	Re E 1b	Re H 1b
	50	Palermo, Italy (1994)	2	Re E 2a	Re H 3d
	83 (3.74)	Tangier, Morocco (1974)	1	Re E 2a	Re H 3b
	84 (3.75)	Les Sables d'Olonne, France (1975)	1	Re E 2b	Re H 3a
	74 (2.77)	Paris, France (1977)	2	Re E 2c	Re H 3c

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TABLE 1—*Continued*

Species	Strain no.[a]	Origin (yr)	Biogroup	Ribogroup or ribotype	
				*Eco*RV	*Hinc*II
	75 (2.81)	Dakar, Senegal (1981)	3	Re E 2d	Re H 5a
	73 (3.83)	Espalion, France (1983)	2	Re E 2e	Re H 3e
	46	Palermo, Italy (1993)	1	Re E 3a	ND
	76 (1.80)	Argenton-sur-Creuse, France (1980)	1	Re E 3b	Re H 2a
	77 (3.73)	Niort, France (1973)	1	Re E 3b	Re H 2a
	82 (2.79)	Dieppe, France (1979)	2	Re E 3c	Re H 2b
	78 (1.72)	St. Dié, France (1972)	1	Re E 3d	Re H 2c
	79 (1.82)	Alfortville, France (1982)	3	Re E 4a	Re H 4a
	80 (1.76)	Niort, France (1976)	4	Re E 4b	Re H 4a
P. rustigianii	NCTC 11802	Type strain		Ru E 1a	Ru H 1a
	66 (2.88)	Colombes, France (1988)		Ru E 1b	Ru H 2b
	67	CDC 8043.83		Ru E 1b	Ru H 2a
	68 (2.89)	Paris, France (1989)		Ru E 1b	Ru H 2c
	64	CDC 3574.67		Ru E 1c	Ru H 1b
P. heimbachae	NCTC 12003	Type strain		He E 1a	He H 1a
	70	CDC 8055.83		He E 1b	He H 1a
	71	CDC 8054.83		He E 1b	He H 1a
	69	CDC 8056.83		He E 1c	He H 1a
	72	CDC 1519.73		He E 1c	He H 1b

[a] In parentheses is given the SEIP collection number.
[b] ND, not determined.

for band detection and migration measurement, RestrictoTyper was used for interpolation of fragment sizes from migration values by using the Schaffer and Sederoff (24) algorithm, Adanson was used for clustering, and Dendrograf was used for drawing dendrograms. More information on the programs has been published (6) or can be obtained from the author of the Taxotron package (11a). Fragment size was calculated by comparing migration values to the average of the two nearest standard lanes, weighted on the basis of lane proximity. The program RestrictoTyper compared pairs of patterns and calculated a distance coefficient that was the complement of the Dice index. We chose a 4% tolerated error, which means that two fragments were considered identical if their sizes did not differ by more than 4%. A dendrogram was drawn on the basis of the distance matrix generated by the average-linkage algorithm of Bartelemy and Guénoche (1).

RESULTS

Ribotyping of type strains. Among the several restriction endonucleases tested (*Bgl*I, *Bgl*II, *Cla*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Hin*cII, *Mlu*I, *Pst*I, *Pvu*II, *Sac*I, *Sal*I, *Sma*I, *Xba*I, and *Xho*I), *Eco*RV and *Hin*cII were chosen for their ability to provide highest differentiation of genera, species, and strains.

Visual inspection of *Eco*RV-digested DNA from the type strains of the 10 different species of the genera *Proteus*, *Morganella*, and *Providencia* showed remarkably different patterns for each strain (Fig. 1). The number of bands varied from 4 in *P. rettgeri* NCTC 11801 to 13 in *P. rustigianii* NCTC 11802. *P. vulgaris* ATCC 29905 showed seven bands, while *P. vulgaris* NCTC 4175, *P. penneri* NCTC 12737, *P. myxofaciens* NCIMB 13273, and *P. alcalifaciens* NCTC 10286 showed six bands, but the sizes of the fragments differed. Eight bands were given by *P. stuartii* NCTC 11800, 9 by *P. mirabilis* NCTC 11938, 10 by *M. morganii* NCTC 235, and 11 by *P. heimbachae* NCTC 12003. The genetic distances between the type strains of the 10 species have been calculated and are shown by the dendrogram in Fig. 2. After *Hin*cII digestion as well, the observed patterns differed for each type strain by the number and/or the size of the bands (Fig. 3). Each pattern contained from 12 (*P. alcalifaciens* NCTC 10286) to 16 (*P. stuartii* NCTC 11800 and *P. penneri* NCTC 12737) fragments. Four type strains, *P. myxofaciens* NCIMB 13273, *P. heimbachae* NCTC 12003, *P. vulgaris* ATCC 29905, and *P. vulgaris* NCTC 4175, showed 13 bands, but all

profiles, including those of the old and the new *P. vulgaris* type strains, were remarkably different. The *M. morganii* NCTC 235 pattern contained 15 bands, whereas the *P. mirabilis* NCTC 11938, *P. rettgeri* NCTC 11801, and *P. rustigianii* NCTC 11802 patterns had 14 bands. Genetic relatedness among the 10 species is presented in the dendrogram in Fig. 4. With both enzymes, the considerable differences in the number and position of the restriction fragments in the patterns permitted calculation of large genetic distances between the type strains.

rDNA restriction patterns of clinical strains. The patterns revealed by the enzyme *Eco*RV by each strain belonging to the different species and the dendrogram obtained are shown in Fig. 5. Each pattern was designated by the first two letters of the specific epithet and the first letter of the endonuclease used, with a number differentiating ribogroups in a species, followed by a lowercase letter differentiating ribotypes within a ribogroup. In general, the profiles observed for each species agreed with or were very similar to those of the corresponding type strain. Depending on the number and size of the bands observed among clinical strains, 17 ribogroups could be defined. Strains of *P. mirabilis*, *P. penneri*, *M. morganii*, *P. rustigianii*, and *P. heimbachae* had profiles identical or very similar to those of their respective type strains. Strains belonging to other species showed a considerable variability in their patterns, so that distinct ribogroups could be defined. Two ribogroups were observed in *P. alcalifaciens*, four in *P. rettgeri*, and two in *P. stuartii*. The type strains for these three species belonged to ribogroups A1 E 1, Re E 1, and St E 1, respectively (Table 1). The *P. vulgaris* restriction patterns were grouped into two ribogroups, the first one comprising the new type strain, ATCC 29905. The pattern shown by the former *P. vulgaris* type strain, NCTC 4175, differed considerably from both *P. vulgaris* ribogroups as well as from any other strain in this study. Minor differences were frequently observed among patterns of strains belonging to the same ribogroup. These differences defined ribotypes within ribogroups. The numbers of ribogroups and ribotypes identified among the clinical strains in the different species are reported in Table 1.
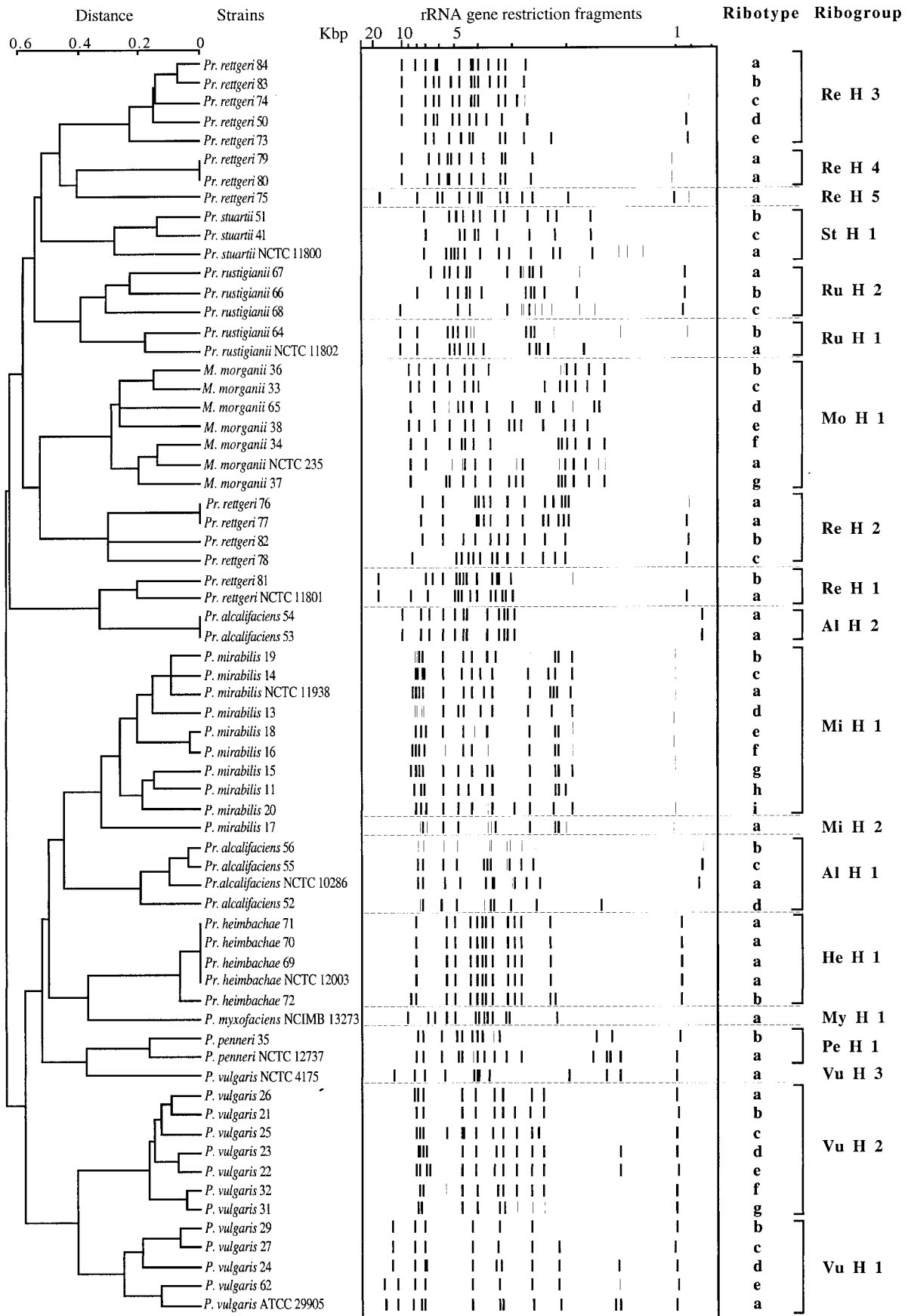
FIG. 6. Dendrogram based on the *Hinc*II restriction patterns of 66 strains listed in Table 1. The distance matrix was generated by using the complement of Dice similarity coefficients and the average-linkage algorithm with a 4% tolerated error.

To verify whether the diversity of patterns observed with *Eco*RV had some taxonomic significance, DNAs of 66 strains were digested with *Hinc*II. The results shown in Fig. 6 confirmed the groupings of strains by *Eco*RV ribotyping. On the other hand, a greater diversity of ribotypes within each species was observed with *Hinc*II.

Biochemical typing. On the basis of the different biochemical patterns, the new type strain ATCC 29905 and seven clinical strains of *P. vulgaris* have been referred to biogroup 2, while the ancient type strain as well as four other clinical strains belonged to biogroup 3. All of the strains of *P. alcalifaciens* and all the strains of *P. stuartii* belonged, respectively, to biogroups 1 and 5 of Ewing et al. (8). Six strains of *P. rettgeri* belonged to biogroup 1, six to biogroup 2, two to biogroup 3, and one to biogroup 4 on the basis of rhamnose and salicin fermentation (23). Strains of the same biogroup belonged to different ribogroups and ribotypes and vice versa, so that no correspondence between biochemical types and ribotypes was shown.

DISCUSSION

Ribotyping has been proposed as a tool for both taxonomic and epidemiological purposes (9). The usefulness of this molecular typing method has been demonstrated when applied to the study of several bacterial genera and species (11, 15, 16, 27). The so-called *Proteus-Providencia* group includes three genera and 10 species that show different degrees of similarity. Cluster analysis of the rRNA gene restriction patterns was performed with both collection and clinical strains belonging to the 10 species and allowed us to define ribogroups for each of the two enzymes used (*Eco*RV and *Hinc*II). In general, a good correlation was observed between ribogroups and nomenspecies. In fact, with both enzymes, no ribogroup included strains belonging to different species. *P. mirabilis*, *P. penneri*, *M. morganii*, *P. rustigianii*, and *P. heimbachae* were confirmed as discrete and homogeneous species, since each species constituted a single ribogroup. In particular, patterns of *P. rustigianii*, previously known as biogroup 3 of *P. alcalifaciens* (14), were clearly distinguishable from those of *P. alcalifaciens*. In fact, two different ribogroups were found in our *P. alcalifaciens* strains. *P. vulgaris* was also divided previously into three biogroups. At present it consists of only two biogroups, 2 and 3, since biogroup 1 now constitutes the separate species *P. penneri*. The digestion patterns of the *P. penneri* strains were widely different from those of *P. vulgaris* strains. Strains of biogroups 2 and 3 of *P. vulgaris* were grouped in two different ribogroups without any correlation with their biochemical behavior. Different ribogroups were also detected among the *P. stuartii* strains (two ribogroups) and *P. rettgeri* strains (four ribogroups), as an expression of their genetic diversity. Actually, genetic diversity had already been observed in some of these species with DNA hybridization studies. The occurrence of at least two relatedness groups had already been demonstrated in *P. rettgeri* (4), and four genomospecies had been described in *P. vulgaris* biogroup 3 (5), whereas biogroup 2 strains of *P. vulgaris* have been shown to be homogeneous by DNA hybridization (13). Our results provide further data on the lack of genetic homogeneity of some species. Moreover, our data support the proposal to substitute *P. vulgaris* NCTC 4175 with strain ATCC 29905 as the type strain for the species. Strain NCTC 4175 is a biogroup 3 strain and, according to DNA-DNA hybridization studies, is a member of genomospecies 3, which, so far, contains only two strains (5). Accordingly, NCTC 4175 showed unique restriction patterns with both enzymes, resulting in an aberrant position for this strain in both

ribotype dendrograms. This result confirms the ability of ribotyping to point out aberrant strains (6). On the contrary, the newly proposed type strain ATCC 29905 (5), belonging to biogroup 2, which contains typical *P. vulgaris* strains, produced with both enzymes restriction patterns clustering together with ribogroup Vu E 1/Vu H 1 strains. In each ribogroup, strains displaying the same *Eco*RV ribotype also displayed closely related *Hinc*II rRNA ribotypes. However, *Hinc*II was more discriminative than *Eco*RV, because it generated more ribotypes than *Eco*RV. The large number of ribotypes observed in a relatively restricted number of strains could suggest a long evolutionary history in the association of these bacteria with humans and animals, resulting in the differentiation of many genetic types from the primary bacterial clones at the origin of the different species. In consideration of the homogeneity of its rDNA-digested patterns, *P. penneri* could be the species most recently differentiated within the *Proteus-Providencia* group.

Bacteria belonging to some species of the *Proteus-Providencia* group are common opportunistic pathogens and are responsible for both hospital- and community-acquired infections in humans. The genetic relatedness of the *P. mirabilis* clinical strains had already been studied by ribotyping, and the results agreed with those of DNA fingerprinting by the arbitrarily-primed PCR technique, permitting demonstration of mother-to-infant vertical transmission of *P. mirabilis* infection (3). The great variability of rDNA restriction patterns observed in almost all of the species studied indicates that ribotyping can be a very sensitive method for epidemiological investigations when applied to the study of infections caused by *Proteus*, *Morganella*, and *Providencia* bacteria. The combination of ribotyping and biotyping can further improve the possibility of strain characterization to detect sources and routes of infection, especially in outbreaks of hospital-acquired infections. Moreover, the correspondence we observed between ribogroups and nomenspecies suggests the potential usefulness of ribotyping for species identification, but for this purpose, a large database of restriction profiles would be required.

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