16S rRNA Gene Sequence Analysis Relative to Genomovars of *Pseudomonas stutzeri* and Proposal of *Pseudomonas balearica* sp. nov.

ANTONIO BENNASAR,^{1*} RAMON ROSSELLÓ-MORA,¹ JORGE LALUCAT,¹
AND EDWARD R. B. MOORE²

Laboratorio de Microbiología, Departamento de Biología Ambiental, Universitat de les Illes Balears, and Institut d'Estudis Avançats (CSIC-UIB), 07071 Palma de Mallorca, Spain,¹ and Bereich Mikrobiologie, Gesellschaft für Biotechnologische Forschung, 38124 Braunschweig, Germany²

We compared the 16S rRNA gene sequences of 14 strains of *Pseudomonas stutzeri*, including type strain CCUG 11256 and strain ZoBell (= ATCC 14405), which represented the seven *P. stutzeri* genomovars (DNA-DNA similarity groups) that have been described. Our sequence analysis revealed clusters which were highly correlated with genomovar clusters derived from DNA-DNA hybridization data. In addition, we identified signature nucleotide positions for each genomovar. We found that the 16S rRNA gene sequences of genomovar 6 strains SP1402^T (T = type strain) and LS401 were different enough from the sequence of the type strain of *P. stutzeri* that these organisms should be placed in a new species, *Pseudomonas balearica*. The type strain of *P. balearica* is strain SP1402 (= DSM 6083).

Pseudomonas stutzeri is a widely distributed, nonfluorescent, denitrifying microorganism that belongs to the authentic genus *Pseudomonas* in the gamma subclass of the *Proteobacteria* (24). *P. stutzeri* is also one of the most controversial bacterial species. Several studies performed during the last few decades have demonstrated that *P. stutzeri* comprises a heterogeneous set of strains that could be placed in more than one species (11, 16, 18). Separation of *P. stutzeri* strains into several species has been attempted, although the extreme phenotypic diversity of the presumptive species did not permit workers to establish definitive differentiating phenotypic criteria, even after exhaustive biochemical analyses (11, 16, 18, 20, 25). Previously, *P. stutzeri* has been reported to include at least seven genomic groups without taxonomic status, which have been called genomovars (18). The results of high-resolution methods, such as chemotaxonomic total fatty acid analysis, total protein pattern analysis, and macrorestriction fragment analysis of genomic DNA, have been consistent with the genomovar subdivisions observed (17, 20).

It has been established that rRNA and their genes, particularly the small-subunit rRNA (16S rRNA), are molecules that can be used to estimate bacterial phylogenetic relationships. In order to clarify the taxonomic positions and the phylogenetic relationships of the *P. stutzeri* genomovars, we determined nearly complete sequences of the 16S rRNA genes of 14 strains representing all genomovars of *P. stutzeri*. For genomovars represented by more than one strain, two or three strains were analyzed. Our results supported the genomovar structure of *P. stutzeri*, confirmed that strain ZoBell is a member of genomovar 2 (19), and showed that genomovar 6 is sufficiently different from the other *P. stutzeri* strains that it should be considered a member of a separate species. We propose the name *Pseudomonas balearica* sp. nov. for the strains of genomovar 6.

* Corresponding author. Mailing address: Laboratorio de Microbiología, Departamento de Biología Ambiental, Universitat de les Illes Balears, and Institut d'Estudis Avançats (CSIC-UIB), Carretera de Valldemossa km 7.5, 07071 Palma de Mallorca, Spain. Phone: (34 71) 173140. Fax: (34 71) 173184. Electronic mail address: DBATBF4@ps.uib.es.

MATERIALS AND METHODS

Strains and culture conditions. The 14 reference strains used in this study have been characterized physiologically and genomically previously (18–20). These strains were originally obtained from culture collections or were isolated as indicated in Table 1. All of the strains were grown in Luria-Bertani broth (22) at 30°C with shaking and were examined to determine their maximum growth temperature (41, 44, or 46°C) and tolerance to NaCl (6, 7, 8, or 9% [wt/vol]).

Isolation of genomic DNA. Cells were harvested at approximately the late exponential phase of growth by centrifugation, washed, and resuspended in 560 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]). Cells were lysed adding 0.1 mg of proteinase K (Sigma) and 30 µl of 10% sodium dodecyl sulfate and incubating the resulting preparation for 1 h at 37°C. DNA was extracted by using the CTAB (hexadecyltrimethylammonium bromide) miniprep protocol for preparation of bacterial genomic DNA (27).

PCR amplification of rRNA genes. The 16S ribosomal DNA was amplified by the PCR by using standard protocols (21) and a Hans Landgraf model 5.92 thermocycler as described previously (8). The forward primer, primer 16F27 (5'-AGAGTTTGATCMTGGCTCAG-3'), annealed at positions 8 to 27, and the reverse primer, primer 16R1488 (5'-CGGTTACCTTGTTAGGACTTCACC-3'), annealed at the complement of positions 1511 to 1488 (*Escherichia coli* numbering [3]).

Sequencing of PCR-amplified DNA. Amplified PCR products were extracted with chloroform-isoamyl alcohol (24:1) and purified with a Microcon-100 microconcentrator (Amicon). Each sample was resuspended in sterile water to a volume of 100 µl, and 5 µl of this preparation was used to check the quality of the PCR-amplified DNA; this was accomplished by performing electrophoresis on a 1% agarose gel with TAE and then staining the gel with ethidium bromide. The sequence of the PCR-amplified 16S ribosomal DNA was determined directly with model 373A automated DNA sequencer (Applied Biosystems, Inc.) by using the protocols recommended by the manufacturer for *Taq* DNA polymerase-initiated cycle sequencing reactions with fluorescently labeled dideoxynucleotide terminators. The primers used for 16S rRNA gene sequence determinations have been described previously (9).

Sequence data analysis. The sequences which we obtained were aligned with reference 16S rRNA sequences by using conserved primary sequence and secondary-structure characteristics as references (6, 28). Evolutionary distances were calculated from sequence pair comparisons as corrected (7) estimates of the average number of fixed-point substitutions per sequence position in homologous sequences since the sequences diverged. Phylogenetic trees were constructed by using subsets of data that included representative sequences of *Pseudomonas* spp. strains (10, 13); to do this, we used distance matrix and bootstrapped distance matrix methods as implemented in the programs of the PHYLIP (4) and ARB (26a) program packages, respectively.

Nucleotide sequence accession numbers. The nucleotide sequences which we determined have been deposited in the GenBank nucleic acid sequence database under the accession numbers shown in Table 1.

TABLE 1. Strains used in this study

Strain ^a	Genomovar	Other designation(s) ^a	Source	G+C content (mol%) ^b	16S ribosomal DNA sequence accession no. ^c
ATCC 17589	1	Stanier 222	Clinical	64.5	U25432
CCUG 11256 ^T	1	ATCC 17588 ^T , Stanier 221 ^T	Clinical	65.0	U26262
ATCC 17591	2	Stanier 224	Clinical	61.4	U26261
ATCC 17587	2	Stanier 220	Clinical	62.3	U25431
ZoBell	2	ATCC 14405	Marine	62.0	U26420
DSM 50227	3	ATCC 11607	Clinical	63.7	U26415
AN10 ^d	3		Marine	63.4	U22427
AN11 ^d	3		Marine	63.3	U25280
19SMN4 ^d	4	DSM 6084	Marine	62.2	U22426
ST27MN3 ^d	4		Marine	62.2	U26419
DNISP21 ^d	5	DSM 6082	Wastewater	63.4	U26414
SP1402 ^{Td}	6	DSM 6083 ^T	Wastewater	64.1	U26418
LS401 ^d	6		Marine	64.4	U26417
DSM 50238	7	ATCC 17832, Stanier 419	Soil	64.9	U26416

^a ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection of the University of Göteborg, Göteborg, Sweden; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; Stanier, R. Y. Stanier et al. (see reference 25).

^b Data from references 2 and 18.

^c GenBank nucleic acid sequence database accession number.

^d Isolated by Rosselló et al. (18).

RESULTS AND DISCUSSION

PCR amplification of the 16S rRNA genes between positions 27 and 1488 allowed us to determine the sequence of 1,456 nucleotide bases (approximately 95% of the complete gene).

The evolutionary distances derived from comparisons of the 16S ribosomal DNA sequences of *P. stutzeri* strains and other members of the genus *Pseudomonas* are shown in Table 2. These data confirmed that all of the strains which we studied belonged to the genus *Pseudomonas* sensu stricto of Palleroni's RNA group I (15) and the gamma subclass of the *Proteobacteria* (24).

The results of our sequence comparisons also supported the

genomovar organization of the species *P. stutzeri*. The evolutionary distances between strains belonging to the same genomovar were always less than 0.46, whereas no evolutionary distance between any *P. stutzeri* strain and another *Pseudomonas* species was less than 2.23 (the evolutionary distance between genomovar 3 strains and *Pseudomonas putida* strains). The largest evolutionary distance observed between *P. stutzeri* strains was 4.44 (the evolutionary distance between genomovar 6 strain LS401 and genomovar 3 strain AN10). Figure 1 is a phylogenetic tree which shows the inferred phylogenetic relationships of *P. stutzeri* strains, as well as other RNA group I *Pseudomonas* species.

TABLE 2. Evolutionary distances for all of the *P. stutzeri* strains studied and other *Pseudomonas* species and ΔT_m values

Strain	Evolutionary distance or ΔT_m value (°C) ^a																	
	<i>P. aeruginosa</i> DSM 50071 ^T	<i>P. balearica</i> LS401	<i>P. balearica</i> SP1402 ^T	ATCC 17589	CCUG 11256 ^T	DN5P21	DSM 50238	AN10	AN11	DSM 50227	19SMN4	ST27/MN3	ATCC 17587	ATCC 17591	ZoBell	<i>P. putida</i> DSM 291 ^T	<i>P. fluorescens</i> B62	<i>P. mendocina</i> DSM 50017 ^T
<i>P. aeruginosa</i> DSM 50071 ^T		6.62	6.54	4.66	4.66	4.74	4.90	5.80	5.80	5.88	5.31	5.31	5.39	5.47	5.47	6.37	6.00	7.07
<i>P. balearica</i> LS401			0.38	3.65	3.65	3.57	4.36	4.44	4.36	4.36	4.04	4.04	4.12	4.20	4.12	5.74	5.82	7.59
<i>P. balearica</i> SP1402 ^T	10.4	0.7		3.57	3.57	3.49	4.28	4.36	4.28	4.28	3.96	3.96	4.04	4.12	4.04	5.66	5.74	7.51
ATCC 17589	11.6	9.4	9.1		0.30	0.38	1.53	2.15	2.07	2.07	1.61	1.61	1.68	1.76	1.76	3.49	3.33	5.12
CCUG 11256 ^T				0.0		0.38	1.53	2.15	2.07	2.07	1.61	1.61	1.68	1.76	1.76	3.49	3.33	5.12
DN5P21			9.2	4.8			1.45	2.07	1.99	1.99	1.53	1.53	1.61	1.68	1.68	3.41	3.25	5.20
DSM 50238	9.4	7.8	8.4	8.8		8.1		2.46	2.46	2.38	1.92	1.92	1.84	1.92	1.92	3.96	3.65	5.12
AN10				8.5					0.46	0.46	1.68	1.68	1.61	1.61	1.68	2.38	3.41	4.15
AN11				7.5						0.46	1.68	1.68	1.61	1.61	1.68	2.23	3.41	4.15
DSM 50227	13.5	10.8	10.1	8.3		7.6	8.2	1.2	1.2		1.61	1.61	1.53	1.53	1.61	2.38	3.33	4.15
19SMN4			9.4	8.3		8.7	9.1	7.0		6.7		0.30	0.53	0.61	0.61	3.25	2.62	4.79
ST27/MN3				7.9						6.4	0.2		0.53	0.61	0.61	3.25	2.62	4.79
ATCC 17587				8.8						6.9				0.38	0.38	3.17	2.70	4.71
ATCC 17591	13.3	11.4	10.8	8.2	7.7	7.8	9.2	6.4	6.4	6.8	7.5	6.7	0.0		0.46	3.17	2.78	4.71
ZoBell	15.1		11.7			8.4	10.9			5.9	7.2			0.6		3.25	2.78	4.79
<i>P. putida</i> DSM 291 ^T																	2.93	4.79
<i>P. fluorescens</i> B62																		5.38
<i>P. mendocina</i> DSM 50017 ^T																		

^a The values on the upper right are evolutionary distances, and the values on the lower left are ΔT_m values (obtained from reference 18).

FIG. 2. Secondary structure of helix 6-V1 in *E. coli* 16S rRNA (14) and the proposed helices for *P. stutzeri* CCUG 11256^T and genomovar 6. Helix 6-V1 is considered proven by comparative analysis (sequences or oligonucleotides catalog). The *E. coli* numbering is the numbering in reference 3. Canonical pairs are connected by lines; G·U pairs are connected by solid circles; and other noncanonical base pairs are connected by open circles.

tances, 0.38 to 0.61) did not correlate with the relatively high ΔT_m values (4.8 to 7.5°C). The correlations between ΔT_m and evolutionary distance data which we observed are shown in Fig. 3. Our results showed that except for genomovar 4 and 5 strains evolutionary distances were highly correlated with ΔT_m values. Thus, *P. stutzeri* genomovar 4 and 5 strains can be added to the exceptional taxa in which 16S sequence similarity

data and DNA-DNA pairing results are not correlated (1, 5, 12); this provides further evidence that systematic relationships below the genus level can be determined definitively only by using a polyphasic approach.

At this time, we still do not believe that *P. stutzeri* should be divided along the lines of the different genomovars, because of the extreme heterogeneity exhibited by the strains belonging to the individual genomovars (18, 20). Although genomovar is not a recognized taxonomic rank (26b), the *P. stutzeri* genomovars may represent the evolutionary state prior to species differentiation, which recently has been proposed to be correlated with levels of 16S rRNA gene sequence similarity higher than 97% (23).

Genomovar 6 represents a new *Pseudomonas* species. Strains LS401 and SP1402^T (T = type strain) were isolated from Mediterranean marine sediment and from a wastewater treatment plant in Mallorca, Spain, respectively. Both of these strains were initially identified as members of *P. stutzeri* because they had the main phenotypic characteristics that define *P. stutzeri* (15). Strains LS401 and SP1402^T were placed together in the same genomovar (genomovar 6) because of their low ΔT_m value, and this group has been correlated with phenotypic and chemotaxonomic markers (18, 20). Genotypically, genomovar 6 was only distantly related to all of the other *P. stutzeri* genomovars, with DNA-DNA hybridization values (ΔT_m values) ranging from 7.8 to 11.7°C (Table 2). Comparisons of the 16S rRNA gene sequences of strains LS401 and SP1402^T have demonstrated that neither of these strains is not affiliated with *P. stutzeri* sensu stricto and that they represent a separate lineage within the genus *Pseudomonas*. However, their closest relatives (minimum evolutionary distance, 3.49) are *P. stutzeri* strains.

Given the DNA-DNA hybridization and 16S rRNA gene sequence data, the significance of phenotypic characteristics that differentiate the genomovar 6 strains from the *P. stutzeri* branch and other *Pseudomonas* species may be appreciated. The genomovar 6 strains exhibit the same characteristic fatty

FIG. 3. Representation of the correlation found between DNA-DNA similarity values (expressed as ΔT_m in degrees Celsius) and the distance values obtained in the 16S rRNA gene sequence analysis. Both values clearly differentiate strains of genomovar 6 as a new species separated from the rest of the strains of *P. stutzeri*. Strains of genomovar 4 and 5 were the only case in which ΔT_m values (4.8 to 7.5°C) did not correlate with the relatively small evolutionary distances (0.35 to 0.61). These results are indicated with asterisks. Symbols: □, same genomovar (genomovars 1 through 5 and 7); ●, genomovar 6; *, genomovar 1 versus genomovar 5 and genomovar 2 versus genomovar 4; ◆, between other different genomovars; ○, genomovar 6 versus *P. stutzeri* (genomovars 1 through 5 and 7) and *P. aeruginosa*; ◇, *P. stutzeri* (genomovars 1 through 5 and 7) versus *P. aeruginosa*.

TABLE 5. Characteristics that differentiate *P. balearica* from *P. stutzeri*, *P. putida*, and *Pseudomonas aeruginosa*^a

Characteristic	<i>P. balearica</i>	<i>P. stutzeri</i>	<i>P. putida</i>	<i>P. aeruginosa</i>
Hydrolysis of:				
Gelatin	— ^b	—	—	+
Starch	+	+	—	—
Utilization of:				
Maltose	+	+	+/-	—
Xylose	+	—	+/-	—
4-Aminobutyrate	—	+/-	+/-	—
Malate	+	+	—	+/-
Suberate	—	+/-	—	—
Mannitol	—	+/-	—	+
Ethylene glycol	—	+	—	—
Denitrification	+	+	—	+
Growth at:				
42°C	+	+/-	—	+
46°C	+	+/-	—	—
Growth in the presence of 8.5% NaCl	+	—	—	—
Fatty acid content (%)				
17:0c	4.71	0.28–1.72	>5	0.8
19:0c	3.8	0.32–1.45	tr	1.2
G+C content (mol%)	64.1–64.4	60.9–64.9	60.7–62.5	67

^a Data from references 15, 18, and 20.^b —, negative; +/-, variable within the group; +, positive traces.

acid profile, which is different from the fatty acid profiles of all other *Pseudomonas* species (26), which contain significant amounts of cyclopropane fatty acids (17:0c and 19:0c). In addition, genomovar 6 strains can be differentiated from *P. stutzeri* strains by their ability to grow at 46°C, their ability to grow in the presence of 8.5% NaCl, and their ability to utilize xylose as a sole carbon source (Table 5). Therefore, we propose that both genomovar 6 strains should be placed in a new species, *Pseudomonas balearica*. The differentiating phenotypic characteristics mentioned above are based on the only two genomovar 6 strains that have been isolated. The characteristics of this limited number of strains may not accurately reflect the internal variation of the species. However, recognition of the new species, isolation of new strains, and exhaustive phenotypic characterization should eventually result in a more accurate description of the phenotype of *P. balearica*.

Description of *Pseudomonas balearica* sp. nov. *Pseudomonas balearica* (ba.le.a'ri.ca.L. fem. adj. *balearica*, of the Balearic Islands, where the organism was isolated). Gram-negative, short, straight, rod-shaped cells that are 0.3 to 0.5 µm wide and 1.5 to 3.0 µm long. Motile by means of a single polar flagellum. Strictly oxidative. Vigorous denitrifier that liberates copious amounts of nitrogen gas from nitrate. Nonpigmented. Has some of the same phenotypic and morphological characteristics as *P. stutzeri*, including the following: amylase and maltose positive, arginine dihydrolase and gelatinase negative, and a characteristic wrinkly dry colony morphology when isolates are fresh. Can be differentiated from *P. stutzeri* by its ability to grow at 46°C and its ability to grow in the presence of 8.5% NaCl, as well as its ability to use xylose as a sole carbon and energy source and its inability to use mannitol, ethylene glycol, 4-aminobutyrate, and suberate. The two strains that have been isolated were isolated as 2-methylnaphthalene degraders at 40°C from wastewater or marine sediment samples.

The G+C content ranges from 64.1 to 64.4 mol%, as determined by high-performance liquid chromatography (18). The type strain is strain SP1402 (= DSM 6083).

ACKNOWLEDGMENTS

This work was supported in part by grants BIO 91-0659 and MAR 91-0341 from CICYT (Spain). A fellowship from the Ministerio de Educación y Ciencia (Spain) to A. Bennasar is gratefully acknowledged. E.R.B.M. was supported in part by European Union HRAMI T-Project grant BIOT-0319-433A and by grant BMFT Vorhaben 0319-433A from the German Ministry of Research and Technology.

We gratefully acknowledge the technical assistance of Angelika Arnscheidt and Annette Krüger. We also thank K. N. Timmis for advice and discussions and for critically reading the manuscript.

REFERENCES

- Ash, C., J. A. E. Farrow, S. Wallbanks, and M. D. Collins. 1991. Phylogenetic heterogeneity of the genus *Bacillus* revealed by comparative analysis of small-subunit-ribosomal RNA sequences. *Lett. Appl. Microbiol.* **13**:202–206.
- Baumann, P., R. D. Bowditch, L. Baumann, and B. Beaman. 1983. Taxonomy of marine *Pseudomonas* species: *P. stutzeri* sp. nov.; *P. perfectomarina* sp. nov., nom. rev.; *P. nautica*; and *P. doudoroffi*. *Int. J. Syst. Bacteriol.* **33**: 857–865.
- Bennasar, A., and J. Lalucat. Manuscript in preparation.
- Brosius, J., M. L. Palmer, P. J. Kennedy, and H. F. Noller. 1978. Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **75**:4801–4805.
- Felsenstein, J. 1982. Numerical methods for inferring phylogenetic trees. *Q. Rev. Biol.* **57**:379–404.
- Fox, G. E., J. D. Wisotzkey, and J. P. Jurtshuk. 1992. How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. *Int. J. Syst. Bacteriol.* **42**:166–170.
- Gutell, R. R., B. Weiser, C. R. Woese, and H. F. Noller. 1985. Comparative anatomy of 16S-like ribosomal RNA. *Prog. Nucleic Acid Res. Mol. Biol.* **32**: 155–216.
- Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules, p. 21–132. In H. N. Munro (ed.), *Mammalian protein metabolism*. Academic Press, Inc., New York.
- Karlson, U., D. F. Dwyer, S. W. Hooper, E. R. B. Moore, K. N. Timmis, and L. D. Eltis. 1993. Two independently regulated cytochromes P-450 in a *Rhodococcus rhodochrous* strain that degrades 2-ethoxyphenol and 4-methoxybenzoate. *J. Bacteriol.* **175**:1467–1474.
- Lane, D. J. 1991. 16S/23S sequencing, p. 115–175. In E. Stackebrandt and M. Goodfellow (ed.), *Nucleic acid techniques in bacterial systematics*. John Wiley & Sons, Chichester, United Kingdom.
- Maidak, B. L., N. Larsen, M. J. McCaughey, R. Overbeek, G. Olsen, K. Fogel, J. Blandy, and C. R. Woese. 1994. The Ribosomal Database Project. *Nucleic Acids Res.* **22**:3485–3487.
- Mandel, M. 1966. Deoxyribonucleic acid base composition in the genus *Pseudomonas*. *J. Gen. Microbiol.* **43**:273–292.
- Martínez-Murcia, A. J., S. Benlloch, and M. D. Collins. 1992. Phylogenetic interrelationships of members of the genera *Aeromonas* and *Plesiomonas* as determined by 16S ribosomal DNA sequencing: lack of congruence with results of DNA-DNA hybridizations. *Int. J. Syst. Bacteriol.* **42**:412–421.
- Moore, E. R. B. Unpublished data.
- Neefs, J. M., Y. Van de Peer, L. Hendriks, and R. De Wachter. 1990. Compilation of small ribosomal subunit RNA sequences. *Nucleic Acids Res.* **18**:2237–2317.
- Palleroni, N. J. 1984. Genus I. *Pseudomonas*, p. 141–199. In N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. Williams & Wilkins Co., Baltimore.
- Palleroni, N. J., R. Kunisawa, R. Contopoulou, and M. Doudoroff. 1973. Nucleic acid homologies in the genus *Pseudomonas*. *Int. J. Syst. Bacteriol.* **23**:333–339.
- Rainey, P. B., I. P. Thompson, and N. J. Palleroni. 1994. Genome and fatty acid analysis of *Pseudomonas stutzeri*. *Int. J. Syst. Bacteriol.* **44**:54–61.
- Roselló, R., E. García-Valdés, J. Lalucat, and J. Ursing. 1991. Genotypic and phenotypic diversity of *Pseudomonas stutzeri*. *Syst. Appl. Microbiol.* **14**: 150–157.
- Roselló-Mora, R. A., E. García-Valdés, and J. Lalucat. 1993. Taxonomic relationship between *Pseudomonas perfectomarina* strain ZoBell and *Pseudomonas stutzeri*. *Int. J. Syst. Bacteriol.* **43**:852–854.
- Roselló-Mora, R. A., J. Lalucat, W. Dott, and P. Kämpfer. 1994. Biochemical and chemotaxonomic characterization of *Pseudomonas stutzeri* genomovars. *J. Appl. Bacteriol.* **76**:226–233.
- Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**:487–491.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Stackebrandt, E., and B. M. Goebel. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* **44**:846–849.

24. **Stackebrandt, E., R. G. E. Murray, and H. G. Trüper.** 1988. *Proteobacteria* classis nov., a name for the phylogenetic taxon that includes the "purple bacteria and their relatives." *Int. J. Syst. Bacteriol.* **38**:321–325.
25. **Stanier, R. Y., N. J. Palleroni, and M. Doudoroff.** 1966. The aerobic pseudomonads: a taxonomic study. *J. Gen. Microbiol.* **43**:159–271.
26. **Stead, D. E.** 1992. Grouping of plant-pathogenic and some other *Pseudomonas* spp. by using cellular fatty acid profiles. *Int. J. Syst. Bacteriol.* **42**: 281–295.
- 26a. **Strunk, O., and W. Ludwig.** Unpublished data.
- 26b. **Ursing, J. B., R. A. Rosselló-Mora, E. García-Valdés, and J. Lalucat.** 1995. Taxonomic note: a pragmatic approach to the nomenclature of phenotypically similar genomic groups. *Int. J. Syst. Bacteriol.* **45**:604.
27. **Wilson, K.** 1987. Preparation of genomic DNA from bacteria, p. 2.4.1.-2.4.2. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), *Current protocols in molecular biology*. John Wiley & Sons, Inc., New York.
28. **Woese, C. R., R. Gutell, R. Gupta, and H. F. Noller.** 1983. Detailed analysis of the higher-order structure of 16S-like ribosomal ribonucleic acids. *Microbiol. Rev.* **47**:621–669.
29. **Zuker, M., and P. Stiegler.** 1981. Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information. *Nucleic Acids Res.* **9**:133–148.