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Phylogeny and Identification of *Pantoea* Species and Typing of *Pantoea agglomerans* Strains by Multilocus Gene Sequencing▽†

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***Pantoea agglomerans* and other *Pantoea* species cause infections in humans and are also pathogenic to plants, but the diversity of *Pantoea* strains and their possible association with hosts and disease remain poorly known, and identification of *Pantoea* species is difficult. We characterized 36 *Pantoea* strains, including 28 strains of diverse origins initially identified as *P. agglomerans*, by multilocus gene sequencing based on six protein-coding genes, by biochemical tests, and by antimicrobial susceptibility testing. Phylogenetic analysis and comparison with other species of *Enterobacteriaceae* revealed that the genus *Pantoea* is highly diverse. Most strains initially identified as *P. agglomerans* by use of API 20E strips belonged to a compact sequence cluster together with the type strain, but other strains belonged to diverse phylogenetic branches corresponding to other species of *Pantoea* or *Enterobacteriaceae* and to probable novel species. Biochemical characteristics such as fosfomycin resistance and utilization of D-tartrate could differentiate *P. agglomerans* from other *Pantoea* species. All 20 strains of *P. agglomerans* could be distinguished by multilocus sequence typing, revealing the very high discrimination power of this method for strain typing and population structure in this species, which is subdivided into two phylogenetic groups. PCR detection of the *repA* gene, associated with pathogenicity in plants, was positive in all clinical strains of *P. agglomerans*, suggesting that clinical and plant-associated strains do not form distinct populations. We provide a multilocus gene sequencing method that is a powerful tool for *Pantoea* species delineation and identification and for strain tracking.**

The genus *Pantoea* includes several species that are generally associated with plants, either as epiphytes or as pathogens, and some species can cause disease in humans. *Pantoea agglomerans*, the *Pantoea* species most commonly isolated from humans, is widely distributed in nature and has been isolated from numerous ecological niches, including plants, water, soil, humans, and animals. It is frequently associated with plants as an epiphyte or an endophyte, and some isolates have been reported to be tumorogenic pathogens (20, 51). As an opportunistic human pathogen, *P. agglomerans* can occur sporadically or in outbreaks. At the beginning of the 1970s, *P. agglomerans* (then called *Enterobacter agglomerans*) was implicated in a large U.S. and Canadian outbreak of septicemia caused by contaminated closures on bottles of infusion fluids; 25 hospitals were involved, with 378 cases (34). Since then, *P. agglomerans* bacteremia has also been described in association with the contamination of intravenous fluid, parenteral nutrition, the anesthetic agent propofol, blood products, and transference tubes used for intravenous hydration (2–4, 22, 36). *P. agglomerans* has been recovered from joint fluids of patients with arthritis, synovitis, or osteomyelitis (7). Infection often occurs after injuries with plant thorns, wood slivers, or wooden splinters (7, 8, 16, 30, 40, 49). Cases of peritonitis due to *P. agglomerans* have also been reported (15, 31). Finally, *P. agglomerans*, which is known to colonize cotton and cotton plants heavily, is associated with cotton fever, a benign febrile syndrome seen in intravenous drug abusers (14).

Seven *Pantoea* species are currently distinguished: *P. agglomerans*, the type species of the genus; *Pantoea ananatis*; *Pantoea stewartii* (divided into the two subspecies *Pantoea stewartii* subsp. *stewartii*, the agent of Stewart's vascular wilt in maize and sweet corn, and *Pantoea stewartii* subsp. *indologenes*); *Pantoea dispersa*; *Pantoea citrea*; *Pantoea punctata*; and *Pantoea terrea* (18, 20, 28, 37). The *Pantoea agglomerans* complex was previously designated *Erwinia herbicola* or *Enterobacter agglomerans* (18). The biochemical heterogeneity of *P. agglomerans* and related strains and species renders identification difficult, even if several biochemical or nutritional characteristics distinguish the "Japanese group" (20) of *Pantoea* species (*P. citrea*, *P. punctata*, and *P. terrea*). Currently, confident identification is not achieved routinely.

Precise knowledge of the phylogenetic relationships and the degree of genetic distinctness among *Pantoea* species is a prerequisite for their correct identification. Phylogenetic relationships among *Pantoea* species were initially based on 16S rRNA analysis (24, 39, 53), which showed that *P. agglomerans*, *P. ananatis*, and *P. stewartii* were closely related. The same result was obtained based on the three protein-

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coding genes *atpD*, *carA*, and *recA* (53). However, only one or a few strains per species were analyzed, and the phylogenetic relationships of the three "Japanese" species with the other *Pantoea* species have, to our knowledge, never been described. Hence, it is not clear whether *Pantoea* species are clearly demarcated and if the genus *Pantoea* forms only one phylogenetic branch.

Defining bacterial species remains a challenge, especially given the fact that homologous recombination or lateral gene transfer can disturb species boundaries. Currently, the approach to defining bacterial species uses both genomic and phenotypic characteristics. The pragmatic values of 70% DNA-DNA reassociation and a difference of <5°C in the melting temperature have been proposed as a cutoff for species definition (50) but are technically challenging to obtain. Multilocus sequence analysis (MLSA) provides an alternative way to define species and to explore sequence discontinuities among them (19). Phylogenetic analysis of concatenated multiple protein-coding genes sometimes allow one to clearly separate species into sequence clusters that can be used to define species, even if borders may be fuzzy for highly recombinogenic species (17, 23). Moreover, the MLSA approach can be used to estimate homologous recombination among species, which is important for determining the reliability of molecular identification based on one or a few genes and for estimating the impact of homologous recombination or lateral gene transfer on the speciation process. So far, no MLSA approach has been reported for *Pantoea* species.

Strain typing and population genetics studies are necessary for epidemiological purposes and to identify strains with important phenotypes such as virulence to plants or humans (11, 47). For example, it is important to determine if *P. agglomerans* strains differ in their abilities to infect humans or to cause specific diseases in plants. Currently, *P. agglomerans* strains can be differentiated using fluorescent amplified fragment length polymorphism (5) or pulsed-field gel electrophoresis (51). However, these methods do not provide unambiguous definition of clones and clonal families, and the results are generally difficult to compare among laboratories. Currently, the amount of diversity within *P. agglomerans* and its population structure are unknown. A widely accepted method for studying strain relationships is multilocus sequence typing (MLST) (33). It consists of sequencing internal portions of several protein-coding genes. This method provides unambiguous and portable data and allows one to compare data worldwide, which is necessary in order to achieve a comprehensive overview of strain diversity and epidemiological distribution (32). In addition, this method is suitable for studying strain phylogeny and allows one to address evolutionary questions at the strain level within species (11). In contrast to MLSA, MLST relies on the comparison of allelic profiles of strains within species, whereas MLSA uses concatenation of gene sequences to define boundaries and phylogenetic relationships between species.

There are few data concerning the susceptibility of *P. agglomerans* to antimicrobial agents. In 1986, Muytjens et al. reported in vitro susceptibility data for eight species of *Enterobacter*, including 27 strains of *E. agglomerans* (38). The strains exhibited very variable susceptibilities to β-lactams, aminoglycosides, and quinolones. Hieng et al. (25) described a case of septicemia due to an *Erwinia herbicola* strain that was resistant to ampicillin, carbenicillin, and cephalothin (cefalotin) and susceptible to other antibiotics usually active on gram-negative bacilli. Cruz et al. (7) reported similar results. No β-lactamase was found among the *Pantoea* species. In 2000, a clinical isolate of *P. agglomerans* recovered from a patient with septic arthritis was reported to be highly resistant to fosfomycin (8).

The objectives of this study were (i) to define the phylogenetic relationships among *P. agglomerans* strains, or strains that may be misidentified as *P. agglomerans*, and other species of *Enterobacteriaceae*; (ii) to characterize *Pantoea* species or phylogenetic clusters biochemically and for their susceptibilities to antimicrobial agents; and (iii) to develop and evaluate MLST for strain discrimination and determination of the population structure of *P. agglomerans*.

MATERIALS AND METHODS

Bacterial strains. A total of 36 strains belonging to the genus *Pantoea* were used. A set of 28 strains initially identified as *P. agglomerans* were gathered from different microbiology laboratories (Tenon, Saint-Antoine, and Saint-Michel hospitals, Paris, France), from the collection of the Biodiversity of Emerging Bacterial Pathogens Unit (Institut Pasteur, Paris, France), and from the Collection de l'Institut Pasteur (CIP). These strains were mainly isolated from clinical samples (Table 1). Strains were identified using API 20E biochemical strips (bioMerieux SA, Marcy-l'Etoile, France), and eight of them were confirmed as *P. agglomerans* sensu stricto based on DNA-DNA hybridization results (6). Eight type strains, corresponding to described species and subspecies of the genus *Pantoea*, were included for comparison (Table 1).

Total-DNA extraction. Bacterial strains were grown on tryptone-casein-soy agar for 24 h at 30°C. DNA was extracted using the Wizard genomic DNA purification kit (Promega, Madison, WI).

PCR amplifications and sequencing. PCR amplification and sequencing of internal portions of the six housekeeping genes *fusA*, *gyrB*, *leuS*, *pyrG*, *rplB*, and *rpoB* were performed with the oligonucleotide primers given in Table 2, using the protocol described previously for *Plesiomonas shigelloides* (44). These genes were selected because they are single-copy-number genes, essential, and present in many bacterial lineages; therefore, they were expected to be present in all members of the *Enterobacteriaceae*. Primers for *fusA*, *leuS*, and *pyrG* were retrieved from Salerno et al. (44), and primers for *gyrB*, *rplB*, and *rpoB* were designed to amplify all species of the *Enterobacteriaceae*. The PCR cycles used for amplification are shown in Table 2. PCR products were directly sequenced after purification by ultrafiltration (Millipore, France) on both strands using the BigDye Terminator ready reaction kit (version 3.1; Perkin-Elmer). Purification was performed by ethanol precipitation. Sequence reaction products were analyzed using an ABI 3730XL automated DNA sequencer. 16S rRNA was amplified and sequenced under the same conditions using the primers and PCR cycles detailed in Table 2. The *repA* gene was amplified by PCR with the previously described primers Rep23220_5 and Rep25101_3 (51). This PCR was carried out using 30 cycles (30 s at 94°C, 30 s at 45°C, and 1 min at 72°C).

Biochemical characteristics. Biochemical tests were performed using the Biotype-100 system (BioMérieux, Marcy-L'Etoile, France) with Biotype medium 1. Positive tests were scored after observation of turbidity or color change following growth in single carbon sources after 48 h and 96 h of incubation at 30°C, according to the manufacturer's instructions. API 20E strips were used in accordance with the manufacturer's instructions (bioMérieux). The tests were all carried out at least in duplicate, and analytical index profiles were determined after 24 h and 48 h of incubation at 37°C. Identification was performed using the API 20E database (version 4.1) with the analytical profile index and with api*web* identification software.

Antimicrobial susceptibility testing. Antimicrobial susceptibility was determined by the disk diffusion method on Mueller-Hinton agar (Bio-Rad, Marnes la Coquette, France) according to the recommendations of the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM) (http://www.sfm.asso.fr). The antibiotic disks (Bio-Rad) were as follows: amoxicillin, amoxicillin-clavulanic acid, ticarcillin, ticarcillin-clavulanic acid, piperacillin, piperacillin-tazobactam, cephalothin, cefoxitin, cefotaxime, ceftazidime, aztreonam, imipenem, gentamicin, tobramycin, netilmicin, amikacin, nalidixic acid, ofloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole, fosfomycin, and colistin. MICs of amoxicillin, amoxicillin-clavulanic acid, cephalothin, cefotaxime, and

TABLE 1. Characteristics of the strains used in this study

Study code	Strain name[a]	Original designation	Original identification method	MLSA identification	Source	ST	*repA* PCR result
PA13	CIP102191	*P. agglomerans*	API 20E	*Enterobacter cowanii*	Food	NC[b]	Negative
PA8	CIP5549	*P. agglomerans*	API 20E	*Enterobacter cowanii*	Growth medium contaminant	NC	Negative
PA1	SM141	*P. agglomerans*	API 20E	*Pantoea agglomerans*	Clinical, Paris, France (Saint-Michel Hospital),	9	Positive
PA11	SA-BAM	*P. agglomerans*	API 20E	*Pantoea agglomerans*	Blood, Paris, France (Saint-Antoine Hospital)	10	Positive
PA12	SA-CHA	*P. agglomerans*	API 20E	*Pantoea agglomerans*	Blood, Paris, France (Saint-Antoine Hospital)	11	Positive
PA14	CIP102282	*P. agglomerans*	API 20E	*Pantoea agglomerans*	Tobacco	12	Positive
PA17	36779	*P. agglomerans*	API 20E	*Pantoea agglomerans*	Blood, Paris, France (Tenon Hospital)	13	Positive
PA18	2263	*P. agglomerans*	API 20E	*Pantoea agglomerans*	Blood, Paris, France (Saint-Antoine Hospital)	14	Positive
PA7	CIPA181	*P. agglomerans*	API 20E	*Pantoea agglomerans*	Blood, France	15	Positive
PA9	CIP82.100	*P. agglomerans*	API 20E	*Pantoea agglomerans*	Cereal, Canada	16	Positive
PA3	4119	*P. agglomerans*	API 20E	*Pantoea agglomerans*	Clinical, Paris, France (Saint-Michel Hospital)	18	Positive
PA4	26301	*P. agglomerans*	API 20E	*Pantoea agglomerans*	Dialysis, Paris, France (Tenon Hospital)	19	Positive
SB545	04A450	*P. agglomerans*	API 20 E	*Pantoea agglomerans*	Blood, Paris, France	20	Negative
SB4002	014230	*P. agglomerans*	DNA-DNA hybridization[d]	*Pantoea agglomerans*	BBPE collection[c]	2	Positive
SB4003	2774-71	*P. agglomerans*	DNA-DNA hybridization	*Pantoea agglomerans*	Leg wound, BBPE collection	3	Positive
SB4004	82-84	*P. agglomerans*	DNA-DNA hybridization	*Pantoea agglomerans*	BBPE collection	4	Positive
SB4005	85-54	*P. agglomerans*	DNA-DNA hybridization	*Pantoea agglomerans*	BBPE collection	5	Positive
SB4006	CUETM85-53	*P. agglomerans*	DNA-DNA hybridization	*Pantoea agglomerans*	Bean plant leaf	6	Positive
SB4008	EL107	*P. agglomerans*	DNA-DNA hybridization	*Pantoea agglomerans*	BBPE collection	7	Positive
SB4009	ICPB EM102	*P. agglomerans*	DNA-DNA hybridization	*Pantoea agglomerans*	Japan	8	Positive
SB4001	0103202	*P. agglomerans*	DNA-DNA hybridization	*Pantoea agglomerans*	BBPE collection	17	Positive
SB3745	CIP57.51^T	*P. agglomerans*	Type strain	*Pantoea agglomerans*	Knee laceration	1	Positive
SB3686	CIP105207^T	*Pantoea ananatis*	Type strain	*Pantoea ananatis*	*Ananas comosus*, Brazil	NC	Negative
SB3620	ATCC 31623^T	*Pantoea citrea*	Type strain	*Pantoea citrea*	Unspecified	NC	Negative
PA16	CIP102701	*P. agglomerans*	API 20E	*Pantoea dispersa*	Human ear, Le Kremlin Bicêtre, France	NC	Positive
SB3687	CIP105207^T	*Pantoea dispersa*	Type strain	*Pantoea dispersa*	*Ananas comosus*, Brazil	NC	Negative
SB3621	ATCC 31626^T	*Pantoea punctata*	Type strain	*Pantoea punctata*	Mandarin (*Citrus reticulata*), Japan	NC	Negative
SB3640	CIP104006^T	*Pantoea stewartii* subsp. *indologenes*	Type strain	*Pantoea stewartii* subsp. *indologenes*	Millet, India	NC	Negative
SB3656	CIP104005^T	*Pantoea stewartii* subsp. *stewartii*	Type strain	*Pantoea stewartii* subsp. *stewartii*	Maize, United States	NC	Negative
SB3746	CIP105600^T	*Pantoea terrea*	Type strain	*Pantoea terrea*	Soil, Japan	NC	Negative
SB546	07A374	*P. agglomerans*	API 20E	Single branch close to *Enterobacter*	Blood, Freiburg, Germany	NC	Negative
PA15	CIP102343	*P. agglomerans*	API 20E	Single branch close to *Kluyvera* genus	Human urine, Algeria	NC	Negative
PA5	6241	*P. agglomerans*	API 20E	Single branch close to *P. agglomerans*	Blood, Paris, France (Saint-Michel Hospital)	21	Positive
SB547	12A277	*P. agglomerans*	API 20E	Single branch close to *P. agglomerans*	Blood, Utrecht, The Netherlands	22	Negative
PA2	1618	*P. agglomerans*	API 20E	Single branch close to *Pantoea* genus	Clinical, Paris, France (Saint-Michel Hospital)	NC	Negative
PA10	CIP107083	*P. agglomerans*	API 20E	Single branch close to *Rahnella aquatilis*	Beetroot (*Beta vulgaris*), Germany	NC	Negative

[a] ATCC, American Type Culture Collection; CUETM, Collection Unité EcoToxicologie Microbienne, Lille, France; ICPB, International Collection of Phytopathogenic Bacteria.
[b] NC, not coded.
[c] BBPE, Biodiversity of Emerging Bacterial Pathogens, Institut Pasteur, Paris, France.
[d] All DNA-DNA hybridization data are from Brenner et al., 1984 (6).

fosfomycin were determined by the standard agar dilution method according to the recommendations of the CA-SFM.

Data analysis. Chromatogram traces were edited and analyzed using BioNumerics, version 5.10 (Applied Maths, Sint-Martens-Latem, Belgium). Each base of the selected template region was confirmed by at least two chromatograms (forward and reverse); if there were ambiguities for a sequence, an additional sequence reaction was performed. Sequences were aligned with BioNumerics. The best model of nucleotide substitution was determined using the MODELTEST Web server (42). Maximum-likelihood trees and bootstrap values were calculated using PhyML (21) with the appropriate model of nucleotide substitution, and the proportion of invariable sites was estimated. Nucleotide diversity and level of polymorphism were calculated using DNAsp, version 4 (43). MLST data were analyzed by the standard MLST approach: for each gene, an allele number was attributed to each allelic variant, and the sequence type (ST) of a strain corresponded to the combination of the allele numbers of the six genes.

Nucleotide sequence accession numbers. All sequences of MLST genes are available on our publicly accessible MLST Web server (http://www.pasteur.fr

TABLE 2. Primers and conditions used for amplification and sequencing

Gene	Primer name	Primer sequence	Primer positions on coding sequences	PCR cycles	Template size (bp)[a]	Template position on the gene[b]	Template position on the *E. coli* K-12 genome[b]
fusA	fusA3	5′-CAT CGG TAT CAG TGC KCA CAT CGA-3′	36–59	2 min 94°C; 1 min 94°C, 1 min 60°C, 1 min 72°C (10 cycles); 1 min 94°C, 1 min 50°C, 1 min 72°C (21 cycles); 5 min 72°C	633	103–735	3471434–3470802 (RC)
	fusA4	5′-CAG CAT CGC CTG AAC RCC TTT GTT-3′					
gyrB	gyrB3	5′-GCG TAA GCG CCC GGG TAT GTA-3′	57–77	2 min 94°C; 1 min 94°C, 1 min 60°C, 1 min 72°C (10 cycles); 1 min 94°C, 1 min 50°C, 1 min 72°C (21 cycles); 5 min s72°C	417	256–672	3877887–3877471 (RC)
	gyrB4	5′-CCG TCG ACG TCC GCA TCG GTC AT-3′	1488–1508				
	gyrB3i	5′-AAC GCW ATC GAC GAA GC-3′	136–152	Primers used only for sequencing			
	gyrB4i	5′-TGG AAV CCR TCR TTC CAC-3′	771–788				
leuS	leuS3	5′-CAG ACC GTG CTG GCC AAC GAR CAR GT-3′	487–512	2 min 94°C; 1 min 94°C, 1 min 60°C, 1 min 72°C (10 cycles); 1 min 94°C, 1 min 50°C, 1 min 72°C (21 cycles); 5 min 72°C	642	568–1209	673439–672798 (RC)
	leuS4	5′-CGG CGC GCC CCA RTA RCG CT-3′	1274–1293				
pyrG	pyrG3	5′-GGG GTC GTA TCC TCT CTG GGT AAA GG-3′	31–56	2 min 94°C; 1 min 94°C, 1 min 60°C, 1 min 72°C (10 cycles); 1 min 94°C, 1 min 50°C, 1 min 72°C (21 cycles); 5 min 72°C	306	82–387	2907607–2907302 (RC)
	pyrG4	5′-GGA ACG GCA GGG ATT CGA TAT CNC CKA-3′	434–460				
rplB	rplB3	5′-CAG TTG TTG AAC GTC TTG AGT ACG ATC C-3′	227–254	2 min 94°C; 1 min 94°C, 1 min 60°C, 1 min 72°C (10 cycles); 1 min 94°C, 1 min 50°C, 1 min 72°C (21 cycles); 5 min 72°C	333	304–636	3449083–3448751 (RC)
	rplB4	5′-CAC CAC CAC CAT GYG GGT GRT C-3′	685–706				
rpoB	Vic3	5′-GGC GAA ATG GCW GAG AAC CA-3′	1422–1442	4 min 94°C; 30 s 94°C, 30 s 50°C, 30 s 72°C (30 cycles); 5 min 72°C	501	1657–2157	4180924–4181424
	Vic2	5′-GAG TCT TCG AAG TTG TAA CC-3′	2469–2489				
16S rRNA	Ad	5′-AGA GTT TGA TCM TGG CTC AG-3′	8–27	4 min 94°C; 1 min 94°C, 1 min 49°C, 1 min 72°C (35 cycles); 7 min 72°C			
	rJ	5′-GGT TAC CTT GTT ACG ACT T-3′	1492–1510				
	E	5′-ATT AGA TAC CCT GGT AGT CC-3′	787–806	Primers used only for sequencing			
	rE	5′-GGA CTA CCA GGG TAT CTA AT-3′	788–806				
	D	5′-CAG CAG CCG CGG TAA TAC-3′	519–536				

[a] The template corresponds to the internal portion of the PCR product used for sequence comparison.
[b] *E. coli* K-12 was taken as the reference (NCBI genome accession number NC_000913). RC, reverse complement.

/mlst). The *rrs* gene (coding for 16S rRNA) sequences were deposited in the GenBank/EMBL/DDBJ databases under accession numbers FJ357809 to FJ357836.

RESULTS

Phylogenetic relationships. The sequences of internal portions of six protein-coding genes were obtained for the 36 study strains. Phylogenetic analysis of the aligned sequences showed that eight strains were highly divergent, showing less than 85% nucleotide similarity with the remaining strains. Comparison with a reference database containing the sequences of all type strains of *Enterobacteriaceae* showed that these eight strains were closely related to species external to the *P. agglomerans* cluster (Fig. 1). Two strains (PA8 and PA13) were very similar (>99.4%) to *Enterobacter cowanii*, whereas three other strains each formed a single branch close to *Kluyvera intermedia* and *Kluyvera cochleae* (PA15), a cluster of *Enterobacter* species including *Enterobacter cloacae* (SB546), or *Serratia liquefaciens*/*Yersinia pseudotuberculosis* (PA10). The three latter strains potentially represent new species. Finally, the type strains of *P. terrea*, *P. punctata*, and *P. citrea* clustered together with *Tatumella ptyseos*, indicating that these three *Pantoea* species should be reclassified as *Tatumella*.

The remaining 28 strains had at least 86% similarity, on average, to each other and formed a unique branch relative to the type strains of other *Enterobacteriaceae* (Fig. 1). This branch included the type strain of *P. agglomerans* as well as those of the taxa *P. stewartii* subsp. *stewartii*, *P. stewartii* subsp. *indologenes*, *P. ananatis*, and *P. dispersa*. The phylogenetic relationships among the 28 strains were analyzed in detail. With

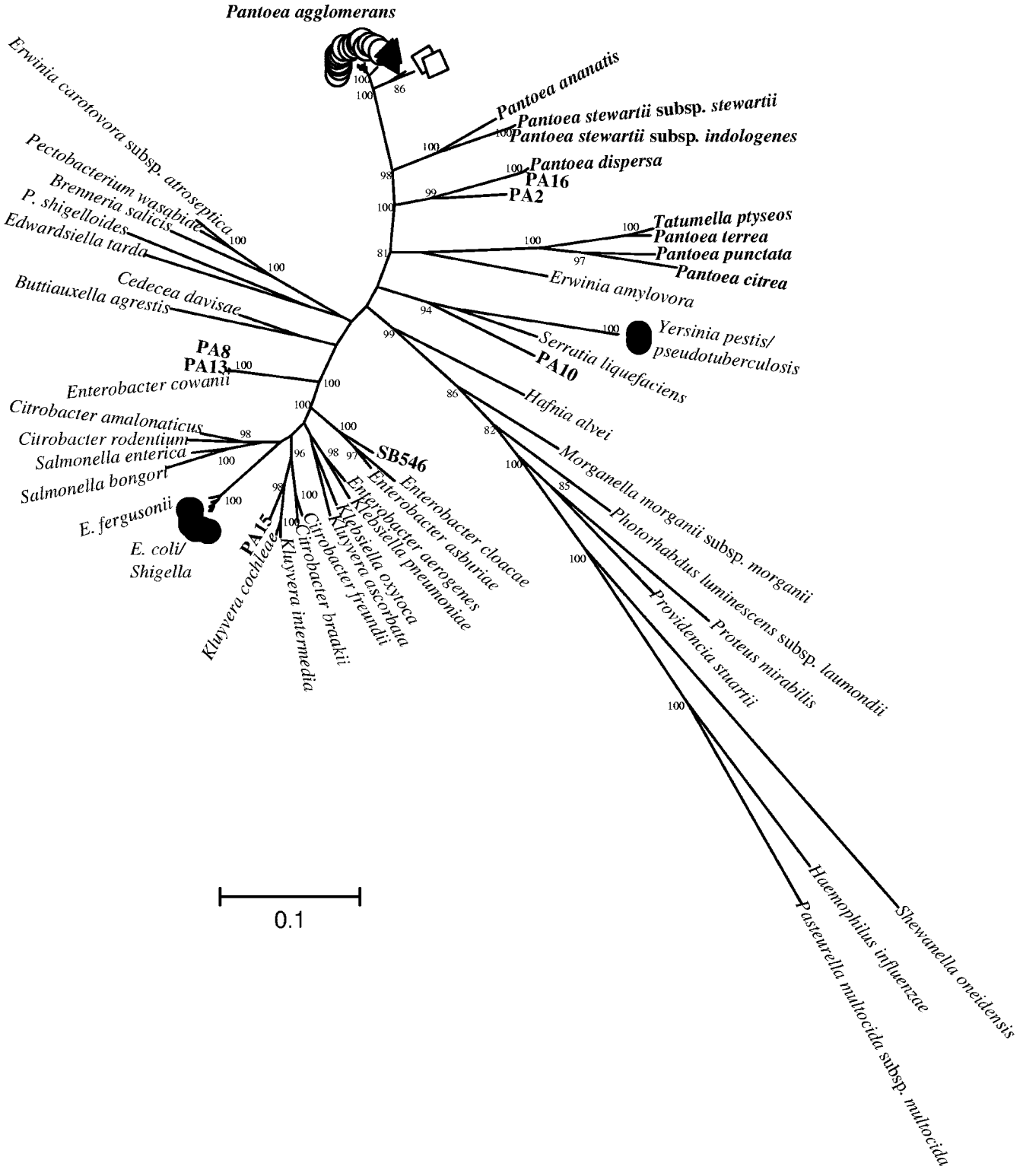

FIG. 1. Maximum-likelihood tree of 34 species of *Enterobacteriaceae* (each represented by its taxonomic type strain), the 36 *Pantoea* study strains, and 3 other gammaproteobacteria, constructed using the concatenated sequences of six loci (*fusA*, *gyrB*, *leuS*, *pyrG*, *rplB*, and *rpoB*). Sequences of the following strains were retrieved from the GenBank database: *Yersinia pestis* CO92 (accession no. NC_003143), *Pasteurella multocida* subsp. *multocida* Pm70 (NC_002663), *Shewanella oneidensis* MR-1 (NC_004347), and *Haemophilus influenzae* Rd KW20 (NC_000907). The numbers at the nodes are bootstrap values higher than 80%, obtained after 1,000 replicates.

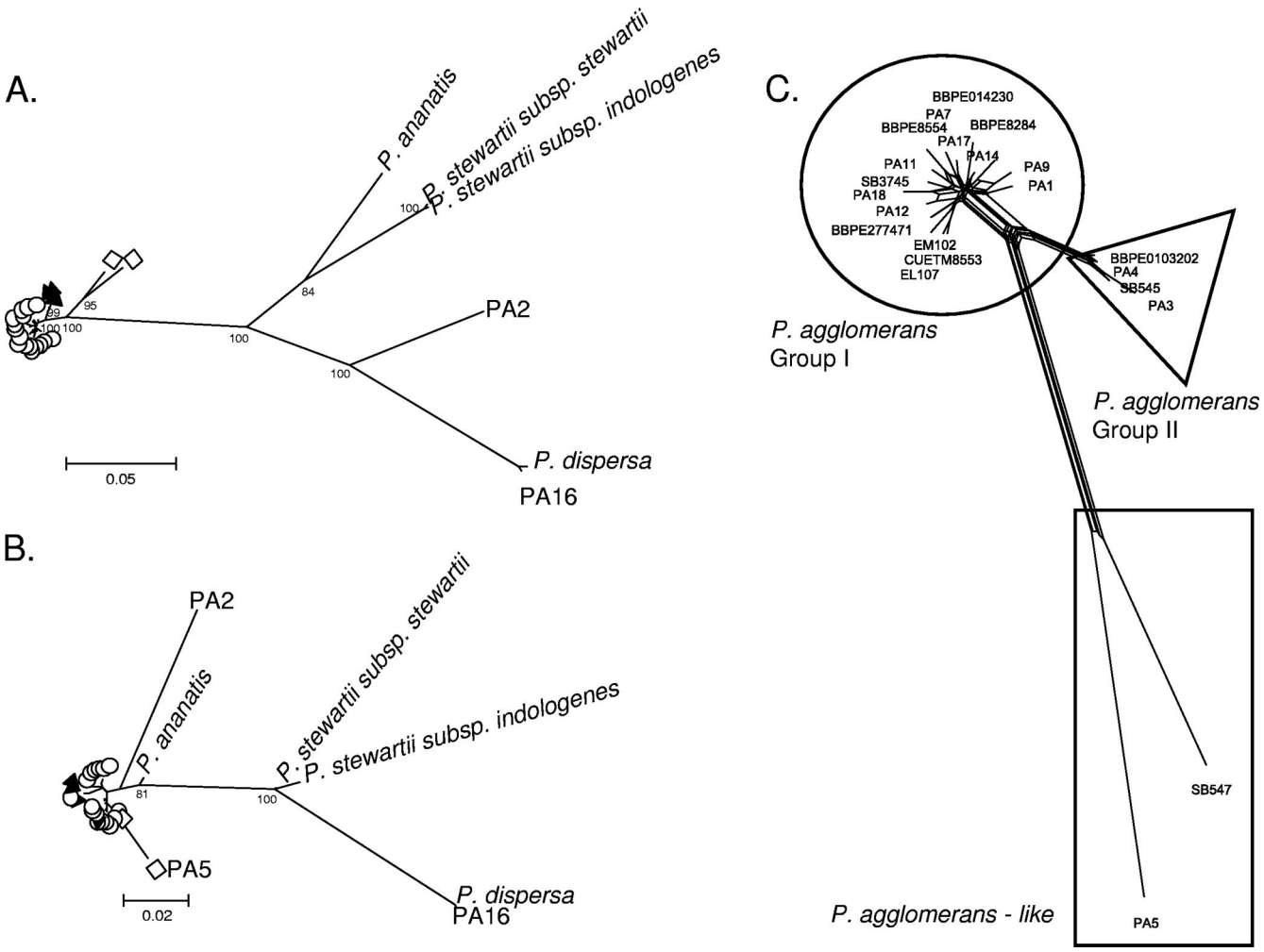

FIG. 2. (A and B) Maximum-likelihood trees of 28 strains of *Pantoea agglomerans* and closely related species, constructed using concatenated sequences of six protein-coding genes (*fusA*, *gyrB*, *leuS*, *pyrG*, *rplB*, and *rpoB*) (A) and 16S rRNA sequences (B) based on the general time-reversible model of nucleotide substitution, which was inferred to be the most likely using MODELTEST. (C) Split decomposition analysis tree of the 20 strains of *P. agglomerans* and 2 *P. agglomerans*-like strains. Circles and triangles represent *P. agglomerans* groups I and II, respectively. The two *P. agglomerans*-like strains, PA5 and SB547, are in the rectangle. The numbers at the nodes are bootstrap values higher than 80%, obtained after 1,000 replicates.

minor exceptions, the individual gene phylogenies of the six protein-coding genes were congruent (see Fig. S1 in the supplemental material). The phylogenies obtained based on the 2,832 bp of the concatenated sequence of the six genes (Fig. 2A) and on 1,491 aligned nucleotides of the 16S rRNA gene (Fig. 2B) are compared in Fig. 2. As expected, the protein-coding genes appeared to evolve much faster than the 16S rRNA gene: the numbers of phylogenetically informative sites were 420 (47.8%) for the concatenate and 46 (3.1%) for 16S rRNA, resulting in increased robustness of the nodes based on protein-coding genes. The minimal similarities observed between two sequences were 86.1% (concatenate) and 95.6% (16S rRNA). Twenty isolates, including the *P. agglomerans* type strain, grouped in a tight cluster with >99% similarity between any pair of strains. Two additional strains, SB547 and PA5, diverged from this *P. agglomerans* cluster by 3.8% and 4.4%, respectively. The taxonomic status of the 2 latter strains is therefore unclear, whereas the 19 isolates that formed a tight cluster with the type strain of *P. agglomerans* can clearly be considered *P. agglomerans*. *P. ananatis* and the two subspecies of *P. stewartii* were associated based on the concatenate (Fig. 2A), whereas *P. ananatis* clustered closer to *P. agglomerans* based on 16S rRNA (Fig. 2B). Finally, strain PA16 was closely related (99.4%) to the type strain of *P. dispersa* and may be identified as belonging this species, whereas strain PA2 formed a unique branch loosely related (89.3%) to *P. dispersa*. Strain PA2 may therefore represent a new species.

Biochemical characterization. In order to assess whether biochemical characteristics can discriminate among species and the phylogenetic clusters identified above, the 36 study strains were characterized biochemically by two systems, API 20E and Biotype-100 strips. Biotype-100 strips test for the ability of strains to grow on 99 carbon sources in minimal medium. Biotype-100 analysis yielded results remarkably consistent with phylogenetic clustering, and some carbon sources distinguished the phylogenetic branches (Fig. 3). In particular,

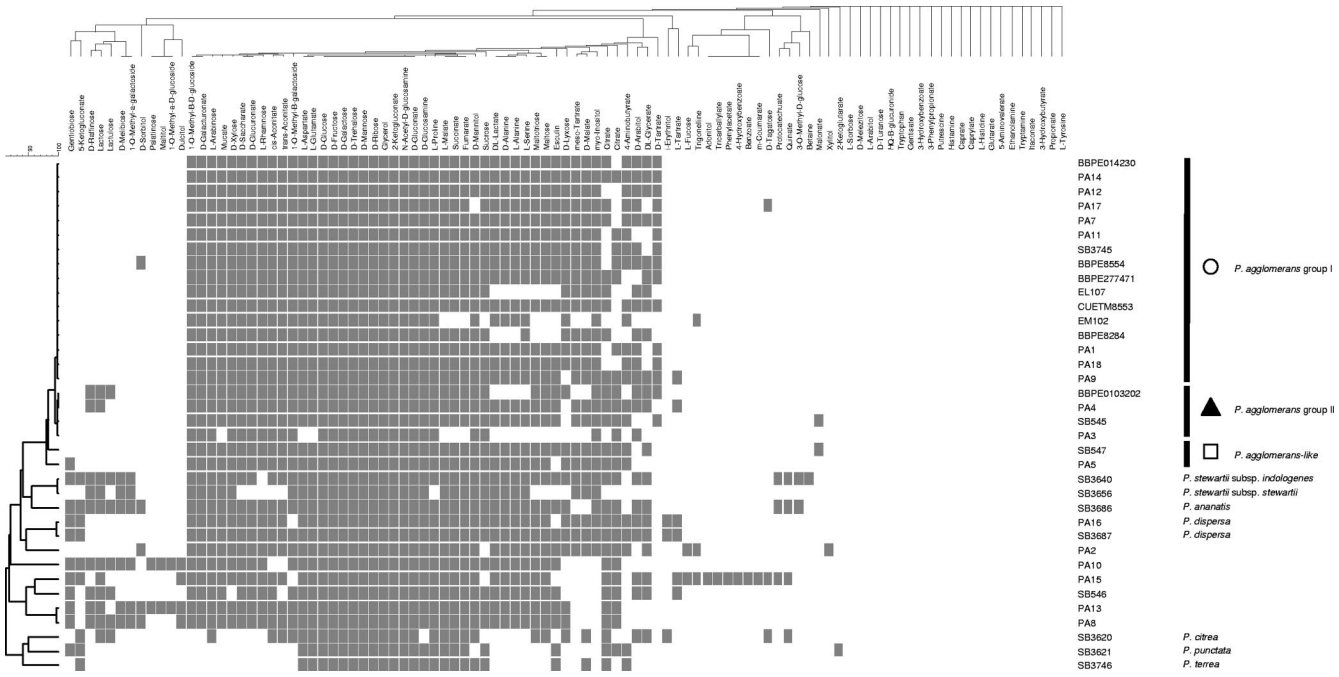

FIG. 3. Biotype-100 results for the 36 strains used in the study. The tree on the left corresponds to a dendrogram, obtained by the unweighted-pair group method using average linkages, based on the concatenated sequences of six protein-coding genes (*fusA*, *gyrB*, *leuS*, *pyrG*, *rplB*, and *rpoB*) for the 36 strains. The tree at the top corresponds to the dendrogram, obtained by the unweighted-pair group method using average linkages, of 99 carbon sources clustered based on Dice similarity. A gray square means that the strain is able to utilize the substrate, whereas an empty square means that the strain is not able to grow on this carbon source after 48 h of incubation.

strains of *P. agglomerans* were the only ones to utilize D-tartrate (75% of strains); *myo*-inositol was used only by *P. agglomerans*, *P. agglomerans*–like strains, and the most closely related species (*P. stewartii*, *P. ananatis*, and *P. dispersa*); and *meso*-tartrate was used by the same group (85% of *P. agglomerans* strains) except for *P. ananatis*. The three divergent *Pantoea* type strains (*P. citrea*, *P. punctata*, and *P. terrea*) did not use a number of substrates, e.g., 1-*O*-methyl-β-D-glucoside and D-galacturonate, which were used by 100% of the other strains. Strain PA15, which clustered in a unique branch based on gene sequences, was unique in its ability to utilize six carbon sources (Fig. 3, adonitol to *m*-coumarate).

Based on API 20E tests, all isolates were typical of the genus *Pantoea*: they were urease negative; lysine and ornithine were not decarboxylated; and H_2S was not produced from thiosulfate. All strains used glucose, mannitol, rhamnose, saccharose, arabinose, and amygdalin as substrates. The variable characteristics are reported in Table 3. Most isolates of *P. agglomerans* produced a yellow pigment on Trypticase soy medium (14/19) and were Voges-Proskauer positive (16/19). Compari-

TABLE 3. Phenotypic characteristics of *Pantoea agglomerans* strains and related strains

Characteristic	Result[a] for:												
	19 *P. agglomerans* strains[b]	*P. agglomerans* CIP57.51^T	SB547	PA5	PA2	PA16	*P. dispersa* CIP103338	*P. ananatis* CIP105207	*P. stewartii* CIP104006	*P. citrea*	*P. punctata*	*P. terrea*	*T. ptyseos*
Yellow pigment	14/19	+	+	−	−	−	+	+	+				
Indole production	0/19	−	−	−	−	−	−	+	+	−	−	−	−
Arginine dihydrolase	0/21	−	+	+	−	−	−	−	−	+	−	−	+
Voges−Proskauer	16/19	+	−	+	+	+	+	+	+	−	+	+	+
Gelatin hydrolysis	19/19[c]	+	+	+	−	−	−	−	+	−	−	−	−
Production of acid from:													
Inositol	0/19 (7/19)[d]	+	−	−	+	−	−	+	−	−	−	−	−
Melibiose	6/19	−	+	−	−	+	+	+	+	+	+	+	−
Fosfomycin susceptibility[e]	0/19	R	S	S	S	S	S	S	S	S	S	S	S

[a] Results are given as positive (+), negative (−), resistant (R), or susceptible (S) for individual strains. For the 19 *P. agglomerans* strains, results are given as the number of strains with the characteristic/total number of strains tested.
[b] Strains PA1, PA3, PA4, PA7, PA9, PA11, PA12, PA14, PA17, PA18, SB4001, SB4002, SB4003, SB4004, SB4005, SB4006, SB4008, SB4009, and SB545.
[c] Gelatin hydrolysis positive in 48 h.
[d] Inositol positive in 48 h.
[e] Determined by the disk diffusion method.

TABLE 4. MICs of several β-lactams

Species or strain(s)	MIC (mg/liter) of the following β-lactam (breakpoints)[a]:			
	Amx (4–16)	Amx-CA (4/2–16/2)	Cf (1–32)	Ctx (4–32)
P. agglomerans (n = 20)	1->128	0.125–2	1–32	<0.06–0.25
SB547	1	1	2	0.125
PA5	1	1	0.5	0.25
P16, *P. dispersa*, *P. stewartii*	1–2	1–2	16–32	0.25
P. ananatis	64	2	1	0.125

[a] Amx, amoxicillin; Amx-CA, amoxicillin-clavulanic acid; Cf, cephalothin; Ctx, cefotaxime. Breakpoints are from the CA-SFM.

son with the phenotypic characteristics of other type strains tested (*P. dispersa*, *P. ananatis*, and *P. stewartii*) showed few differences. Notably, only *P. ananatis* and *P. stewartii* were positive for indole production. The two undefined strains SB547 and PA5 shared the unique characteristic of being arginine dihydrolase positive.

According to the API 20E system database, it was not possible to identify any isolate at the species level. However, the current database includes only *Pantoea* sp1, sp2, sp3, and sp4 (synonyms of *Enterobacter agglomerans* groups 1, 2, 3, and 4). The 19 isolates of *P. agglomerans* were divided into three different numerical codes: 1005173 (8 strains), 1005133 (8 strains), and 1004133 (3 strains), which corresponded, respectively, to *Pantoea* sp3 with 68% confidence, to *Pantoea* sp3 with 99.2% confidence, and to *Pantoea* sp4 with 64% confidence. With the addition of gelatin hydrolysis, which was positive in 48 h for all 19 isolates, these 19 isolates were identified as *Pantoea* sp3 with, respectively, 95%, 99.8%, and 96.4% confidence. Isolate PA16 was identified as *Pantoea* sp2 with only 43% confidence (code 0205173).

Antibiotic susceptibility. All strains of *P. agglomerans* were uniformly susceptible to aminoglycosides (gentamicin, tobramycin, amikacin), fluoroquinolones (ofloxacin, ciprofloxacin), and trimethoprim-sulfamethoxazole. All strains were fully susceptible to broad-spectrum cephalosporins and imipenem. Testing for susceptibility to other β-lactams showed various results (Table 4): nine *P. agglomerans* strains were susceptible to all β-lactams; six strains showed intermediate susceptibility or resistance to amoxicillin; and four strains showed intermediate susceptibility or resistance to cephalothin. Isolates SB547 and PA5 were susceptible to all antibiotics. Strain PA16 and the type strains of *P. dispersa* and *P. stewartii* were resistant to cephalothin, and the type strain of *P. ananatis* was resistant to amoxicillin.

Remarkably, resistance to fosfomycin (MICs, >32 mg/liter) was observed for all *P. agglomerans* strains but for no other species, and may thus provide a useful and simple test for presumptive identification. In particular, isolates SB547 and PA5, which are closely related to *P. agglomerans* based on housekeeping gene sequences, were susceptible to fosfomycin.

MLST discriminates *P. agglomerans* isolates. For the 20 *P. agglomerans* strains and isolates SB547 and PA5, the number of distinct alleles ranged from 4 for *rplB* to 17 for *leuS* (Table 5). Remarkably, each strain had a unique ST, showing that MLST is very discriminatory among *P. agglomerans* strains.

The levels of nucleotide diversity differed greatly among genes (Table 5). The percentage of polymorphic sites ranged from 3% for *fusA* and *rplB* to 14% for *leuS*, while the average number of nucleotide differences per site (π) ranged from 0.3% to 2.6% for *rplB* and *leuS*, respectively. Thus, *P. agglomerans* contains relatively large numbers of nucleotide polymorphisms among orthologous genes, even based on this limited strain sample. No nonsynonymous changes were found in *pyrG* and *rpoB*, and the ratio of synonymous to nonsynonymous changes (K_s/K_a) was higher than 11 for the other genes, consistent with selection against amino acid changes in housekeeping genes.

For microevolutionary studies, phylogenetic relationships among strains using MLST data are typically deduced based on allelic profiles rather than nucleotide sequences, since the former approach is less sensitive to recombination (11). Among *P. agglomerans* isolates, most STs were distant by at least three allelic mismatches, with only two pairs of STs (ST11–ST12 and ST17–ST19) differing by two genes. Thus, it was difficult, based on this restricted sample, to identify groups of closely related STs that could correspond to clonal families.

The phylogenetic structure of *P. agglomerans* was investigated by analysis of nucleotide sequences using SplitsTree (Fig. 2C). This analysis revealed the existence of a cluster of four strains (group II), which was demarcated from the remaining 16 *P. agglomerans* strains, including the type strain (group I). This cluster was also recovered by maximum-likelihood (Fig. 2A) and neighbor-joining (data not shown) phylogenetic reconstructions, as well as from most individual gene phylogenies (see Fig. S1 in the supplemental material). Therefore, two phylogenetic groups can be distinguished within *P.*

TABLE 5. Diversity of six housekeeping genes among 20 *Pantoea agglomerans* and 2 *P. agglomerans*-like strains[a]

Gene	Template size (bp)	No. of alleles	No. (%) of polymorphic sites	π	No. of parsimony informative sites	No. of synonymous changes	No. of nonsynonymous changes	K_s	K_a	K_s/K_a
fusA	633	12	19 (3)	0.00646	9	17	4	0.02269	0.00194	11.7
gyrB	417	15	39 (9.4)	0.01587	15	36	5	0.06244	0.0016	39.0
leuS	642	17	91 (14.2)	0.02617	53	86	7	0.11934	0.00387	30.8
pyrG	306	9	16 (5.2)	0.00633	4	16	0	0.02712	0	NA
rplB	333	4	10 (3.0)	0.00273	1	9	1	0.0096	0.00034	28.2
rpoB	501	13	30 (6.0)	0.00912	15	32	0	0.03836	0	NA
Concatenate	2,832	22	205 (7.2)	0.01233	97	196	17	0.04887	0.0016	30.5

[a] π, average number of nucleotide differences per site for two randomly selected strains; K_s, number of synonymous changes per synonymous site; K_a, number of nonsynonymous changes per nonsynonymous site; NA, not applicable.

agglomerans. Three of four isolates of group II did not utilize D-lyxose, whereas all group I strains except one were positive for this carbon source. Of note, the 16S rRNA gene sequence did not allow separation between the two groups due to its limited amount of polymorphism (Fig. 2B).

Plasmid pPATH is associated with the virulence of *P. agglomerans* for plants (35), and *P. agglomerans* strains differ by the presence of this plasmid (51). Currently, the link between plant strains and human clinical isolates is not known. In order to determine the presence or absence of plasmid pPATH among our strains, we performed a PCR assay (51) that targets the *repA* gene, which codes for the replicase protein A of plasmid pPATH. It appeared that all *P. agglomerans* strains were positive by *repA* PCR, except for strain SB545 of group II. Of the two *P. agglomerans*-like strains SB547 and PA5, the former was negative and the latter was positive. All non-*P. agglomerans* strains were negative except for strain PA16 (identified as *P. dispersa*), which was positive and may have acquired plasmid pPATH horizontally. These results provide no evidence that *P. agglomerans* clinical strains represent a population distinct from plant-pathogenic strains.

DISCUSSION

Universal genes for phylogeny and species borders in *Enterobacteriaceae*. In this work, we have developed and applied multilocus gene sequencing with the combined purposes of determining the phylogenetic relationships among *Pantoea* species and species borders using a MLSA approach and evaluating the usefulness of MLST for *P. agglomerans* strain typing. The primers used here were designed to be applicable to *Enterobacteriaceae* strains of all genera and species and allowed us to successfully amplify and sequence the six genes in species belonging to many genera (Fig. 1). Because this set of genes provides much better resolution than the traditionally used 16S rRNA gene, and because the 16S rRNA gene is not reliable for phylogeny in *Enterobacteriaceae* (39), this set of six protein-coding genes should be useful for future phylogenetic studies of species of *Enterobacteriaceae*.

The six genes *fusA*, *gyrB*, *leuS*, *pyrG*, *rplB*, and *rpoB* were successfully PCR amplified and sequenced for a collection of 36 strains initially identified as belonging to the *Pantoea* genus. The phylogenetic tree based on the concatenated sequences of the six housekeeping genes confirms that the genus *Pantoea* is heterogeneous. *Pantoea* species did not group in a single branch but instead were divided into two clusters. One cluster contained the type species of the genus (*P. agglomerans*), together with *P. ananatis*, *P. dispersa*, and *P. stewartii*. The second cluster contained the species *P. citrea*, *P. terrea*, and *P. punctata*, described by Kageyama et al. (28), which were strongly associated with the type strain of *Tatumella ptyseos*. These results indicate a necessity to revise the taxonomic status of these three species, as was suggested by Grimont and Grimont based on the distinct phenotypic characteristics of the "Japanese" group of *Pantoea* species (20), which possibly should be reclassified as belonging to the genus *Tatumella*.

Within the first cluster, *P. agglomerans* was clearly demarcated from the other species. Indeed, the 19 isolates and the type strain formed a tight cluster with less than 1.5% divergence on average for the six protein-coding genes, while these strains were separated by more than 7% divergence from *P. ananatis* and *P. stewartii*. Therefore, there appears to be no ambiguity in the distinctness of these species based on the present set of strains. In contrast, the two strains SB547 and PA5 showed an intermediate average distance from *P. agglomerans* strains. However, individual gene phylogenies of the six genes do not show the same picture, especially for *fusA*, on the basis of which the two strains clustered inside *P. agglomerans* (group II) (see Fig. S1 in the supplemental material). Some strains can have intermediate positions on phylogenetic trees deduced from concatenated sequences, in particular due to horizontal transfer occurring independently on individual genes, providing conflicting phylogenetic signals. These strains result in unclear demarcation of species, and this difficulty in separating species has led to the concept of "fuzzy species" (23). SB547 and PA5 potentially represent examples of strains that have received DNA sequences from external species, leading to their apparent separation from *P. agglomerans*. Alternatively, they may represent a distinct species that has received some genes (in particular, *fusA*) from *P. agglomerans*. This phenomenon makes it difficult to decide, based on the present data, whether the two strains SB547 and PA5 belong to *P. agglomerans* or to a closely related species. In contrast, PA2 clearly represents a species distinct from all currently described *Pantoea* species, since its uniqueness is supported by all individual gene phylogenies.

It may be noted that the position of *P. ananatis* relative to *P. agglomerans* and *P. stewartii* in the concatenate tree (Fig. 2A) was different from that in the 16S rRNA tree (Fig. 2B). This difference was also apparent in a previous study where 16S rRNA and protein-coding genes were analyzed (53). The plot of the distance based on 16S rRNA versus the distance based on the concatenated sequence showed a strong correlation, with most comparisons fitting on a single line, but *P. ananatis* stood as an outlier, with an atypically high 16S rRNA similarity (data not shown). Therefore, one may suspect that the 16S rRNA gene underwent horizontal transfer between *P. agglomerans* and *P. ananatis*. Based on individual gene phylogenies (see Fig. S1 in the supplemental material), five of our six genes, as well as the *atpD* and *carA* genes (53), grouped *P. ananatis* with *P. stewartii*, while *fusA* grouped *P. ananatis* with *P. agglomerans*. Thus, *fusA* may also have been imported from *P. agglomerans* into *P. ananatis*. Finally, we noted that for both subspecies of *P. stewartii*, the 5′ portion of the 16S rRNA sequence was divergent from those of *P. agglomerans* and *P. ananatis*, with 8 single-nucleotide polymorphisms within 92 nucleotide positions (8.7%), whereas the average divergence over the entire length of the rRNA gene was 2.7%. This observation suggests that the 16S rRNA gene of *P. stewartii* may have a mosaic origin.

Overall, these results illustrate the importance of using multiple independent gene sequences, since they provide much-improved phylogenetic information over that obtained with the 16S rRNA gene. In addition, as proposed in the MLSA approach (23), the use of multiple genes allows one to buffer the effect of horizontal transfer on phylogenies and to be better informed on the validity of assigning strains to species.

Identification tools. Biochemical tests are broadly used for strain identification in clinical laboratories. Our results show that strains identified as *P. agglomerans* by widely used bio-

chemical methods represent a diverse set of strains encompassing several unrelated phylogenetic branches. A majority of isolates initially identified as *P. agglomerans* clustered in a compact group together with the type strain and can be considered as belonging to the species *P. agglomerans*. Nevertheless, the API 20E method led to the misidentification of a few strains (SB546, PA2, PA8, PA10, PA13, PA15, and PA16), since phylogenetic analysis revealed that they did not belong to *P. agglomerans*. Thus, multilocus sequencing of protein-coding genes stands as a useful reference tool for the identification of *P. agglomerans* and for the characterization of atypical strains.

Even though biochemical identification used routinely can be imprecise, the Biotype-100 results were consistent with the phylogeny, and some characteristics appear to be potentially useful and can provide an orientation for strain identification, since they are specific to particular phylogenetic clusters. For example, D-tartrate was used only by *P. agglomerans*, whereas *myo*-inositol and *meso*-tartrate were used only by *P. agglomerans* and closely related *Pantoea* species; these results are consistent with previous data (20). Likewise, resistance to fosfomycin was found to be very useful for the identification of *P. agglomerans*.

Toward a universal MLST scheme for *Enterobacteriaceae*? Strain typing is an important tool for detecting the source of infections in epidemiological investigations (48). In addition, the possible association between ecology or virulence and particular *P. agglomerans* clones is currently unknown, and future research into these important questions would benefit greatly from a standard definition of strains. MLST is now widely recognized as a powerful approach for defining groups of related strains (clones), for determining the geographic and temporal distribution of clones, and for revealing the internal genetic structure of species. Until now, MLST developments have been restricted to a few species of the *Enterobacteriaceae* (1, 9, 29, 52), because it remains difficult to design successful PCR primers for sequencing of protein-coding genes, especially when no genome sequence is available. In addition, because different gene sets are used for different species, and because distinct genes have distinct evolutionary rates, direct comparison of amounts of diversity and population genetics parameters among species is not possible.

We successfully developed an MLST scheme for *Pantoea* species in the absence of a complete genome sequence for *Pantoea* by designing broad-range primers based on universally conserved protein-coding genes. Our data show that MLST is a powerful typing tool for *P. agglomerans*, since each of the 20 isolates had a distinct multilocus genotype (allelic profile). This result was not necessarily expected, since the use of broadly distributed genes could have implied a low evolutionary rate and hence low discrimination. Together with previous results for *Plesiomonas shigelloides* (44) and *Enterobacter cloacae* (41), which also showed high discrimination among strains, the present study therefore indicates that our set of broad-range primers could represent a universal MLST scheme applicable to most, if not all, species of the *Enterobacteriaceae*.

Population structure of *P. agglomerans*. Bacterial species differ widely in the rate of homologous recombination among strains (13, 45). Determining the frequency of recombination is important for understanding the evolution of bacterial pathogens and for the interpretation of typing data (46). Given the relatively small sample and the restricted degree of nucleotide polymorphism, it was not possible to determine the rate of recombination precisely and to demonstrate its occurrence statistically. However, two lines of evidence indicate that recombination is not rare among *P. agglomerans* strains. First, the distribution of nucleotide polymorphisms along the individual phylogenies of the three most informative genes (*gyrB*, *leuS*, and *rpoB*) showed homoplasy, which is likely to result from the occurrence of intragenic recombination in these genes. Second, split decomposition analysis of the concatenated sequences of the six genes showed a network-like structure (Fig. 2C). Typically, networks disclosed upon split decomposition analysis are interpreted as evidence for recombination (26), since they incorporate the effects of both intragenic and intergenic recombination. This analysis, therefore, suggests relatively frequent homologous recombination among *P. agglomerans* strains. This species, therefore, represents another example of a recombining species within the family *Enterobacteriacae*, following the previous demonstration of high rates of recombination in *P. shigelloides* (44) and moderate recombination in *Escherichia coli* (12, 52) and *Salmonella enterica* (10).

Despite evidence for homologous recombination among housekeeping genes, an internal phylogenetic structure could be revealed for *P. agglomerans*. Indeed, we consistently found two groups of strains that were recovered by different analyses and genes. The existence of an internal phylogenetic structure in the presence of recombination is reminiscent of the situation of *E. coli*, where at least six clearly separated groups are distinguished (27). In addition, utilization of D-lyxose appeared to distinguish *P. agglomerans* groups I and II, although this finding should be confirmed with additional strains. There was no association in our limited sample between the groups and the source of isolates, but the study of larger strain collections of diverse origins, including plant-pathogenic isolates and environmental isolates, may reveal associations between phenotypes and multilocus genotypes. In order to provide a common language for *P. agglomerans* strain characterization and evolution, a publicly available MLST website was set up at http://www.pasteur.fr/mlst.

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REFERENCES

1. **Achtman, M., K. Zurth, G. Morelli, G. Torrea, A. Guiyoule, and E. Carniel.** 1999. *Yersinia pestis*, the cause of plague, is a recently emerged clone of *Yersinia pseudotuberculosis*. Proc. Natl. Acad. Sci. USA **96:**14043–14048.
2. **Alvarez, F. E., K. J. Rogge, J. Tarrand, and B. Lichtiger.** 1995. Bacterial contamination of cellular blood components. A retrospective review at a large cancer center. Ann. Clin. Lab. Sci. **25:**283–290.
3. **Bennett, S. N., M. M. McNeil, L. A. Bland, M. J. Arduino, M. E. Villarino, D. M. Perrotta, D. R. Burwen, S. F. Welbel, D. A. Pegues, L. Stroud, et al.** 1995. Postoperative infections traced to contamination of an intravenous anesthetic, propofol. N. Engl. J. Med. **333:**147–154.
4. **Bicudo, E. L., V. O. Macedo, M. A. Carrara, F. F. Castro, and R. I. Rage.** 2007. Nosocomial outbreak of *Pantoea agglomerans* in a pediatric urgent care center. Braz. J. Infect. Dis. **11:**281–284.
5. **Brady, C., S. Venter, I. Cleenwerck, M. Vancanneyt, J. Swings, and T. Coutinho.** 2007. A FAFLP system for the improved identification of plant-pathogenic and plant-associated species of the genus *Pantoea*. Syst. Appl. Microbiol. **30:**413–417.

6. **Brenner, D. J., G. R. Fanning, J. K. Leete Knutson, A. G. Steigerwalt, and M. I. Krichevsky.** 1984. Attempts to classify Herbicola group-*Enterobacter agglomerans* strains by deoxyribonucleic acid hybridization and phenotypic tests. Int. J. Syst. Bacteriol. **34:**45–55.
7. **Cruz, A. T., A. C. Cazacu, and C. H. Allen.** 2007. *Pantoea agglomerans*, a plant pathogen causing human disease. J. Clin. Microbiol. **45:**1989–1992.
8. **De Champs, C., S. Le Seaux, J. J. Dubost, S. Boisgard, B. Sauvezie, and J. Sirot.** 2000. Isolation of *Pantoea agglomerans* in two cases of septic monoarthritis after plant thorn and wood sliver injuries. J. Clin. Microbiol. **38:** 460–461.
9. **Diancourt, L., V. Passet, J. Verhoef, P. A. D. Grimont, and S. Brisse.** 2005. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J. Clin. Microbiol. **43:**4178–4182.
10. **Falush, D., M. Torpdahl, X. Didelot, D. F. Conrad, D. J. Wilson, and M. Achtman.** 2006. Mismatch induced speciation in *Salmonella*: model and data. Philos. Trans. R. Soc. Lond. B **361:**2045–2053.
11. **Feil, E. J.** 2004. Small change: keeping pace with microevolution. Nat. Rev. Microbiol. **2:**483–495.
12. **Feil, E. J., E. C. Holmes, D. E. Bessen, M. S. Chan, N. P. Day, M. C. Enright, R. Goldstein, D. W. Hood, A. Kalia, C. E. Moore, J. Zhou, and B. G. Spratt.** 2001. Recombination within natural populations of pathogenic bacteria: short-term empirical estimates and long-term phylogenetic consequences. Proc. Natl. Acad. Sci. USA **98:**182–187.
13. **Feil, E. J., and B. G. Spratt.** 2001. Recombination and the population structures of bacterial pathogens. Annu. Rev. Microbiol. **55:**561–590.
14. **Ferguson, R., C. Feeney, and V. A. Chirurgi.** 1993. *Enterobacter agglomerans*-associated cotton fever. Arch. Intern. Med. **153:**2381–2382.
15. **Ferrantino, M., S. D. Navaneethan, and J. A. Sloand.** 2008. *Pantoea agglomerans*: an unusual inciting agent in peritonitis. Perit. Dial. Int. **28:**428–430.
16. **Flatauer, F. E., and M. A. Khan.** 1978. Septic arthritis caused by *Enterobacter agglomerans*. Arch. Intern. Med. **138:**788.
17. **Fraser, C., W. P. Hanage, and B. G. Spratt.** 2007. Recombination and the nature of bacterial speciation. Science **315:**476–480.
18. **Gavini, F., J. Mergaert, A. Beji, C. Mielcarek, D. Izard, K. Kersters, and J. De Ley.** 1989. Transfer of *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972 to *Pantoea* gen. nov. as *Pantoea agglomerans* comb. nov. and description of *Pantoea dispersa* sp. nov. Int. J. Syst. Bacteriol. **39:**337–345.
19. **Gevers, D., F. M. Cohan, J. G. Lawrence, B. G. Spratt, T. Coenye, E. J. Feil, E. Stackebrandt, Y. Van de Peer, P. Vandamme, F. L. Thompson, and J. Swings.** 2005. Re-evaluating prokaryotic species. Nat. Rev. Microbiol. **3:**733–739.
20. **Grimont, P. A. D., and F. Grimont.** 2005. Genus *Pantoea*, p. 713–720. *In* D. J. Brenner, N. R. Krieg, and J. T. Staley (ed.), Bergey's manual of systematic bacteriology, 2nd ed., vol. 2. The *Proteobacteria*. Springer-Verlag, New York, NY.
21. **Guindon, S., and O. Gascuel.** 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. **52:**696–704.
22. **Habsah, H., M. Zeehaida, H. Van Rostenberghe, R. Noraida, W. I. Wan Pauzi, I. Fatimah, A. R. Rosliza, N. Y. Nik Sharimah, and H. Maimunah.** 2005. An outbreak of *Pantoea* spp. in a neonatal intensive care unit secondary to contaminated parenteral nutrition. J. Hosp. Infect. **61:**213–218.
23. **Hanage, W. P., C. Fraser, and B. G. Spratt.** 2005. Fuzzy species among recombinogenic bacteria. BMC Biol. **3:**6.
24. **Hauben, L., E. R. Moore, L. Vauterin, M. Steenackers, J. Mergaert, L. Verdonck, and J. Swings.** 1998. Phylogenetic position of phytopathogens within the *Enterobacteriaceae*. Syst. Appl. Microbiol. **21:**384–397.
25. **Hieng, H., C. Richard, J. Marie, and Y. Lemennais.** 1989. A propos d'un cas de septicémie à *Erwinia herbicola*. Med. Mal. Infect. **19:**193–195.
26. **Huson, D. H., and D. Bryant.** 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. **23:**254–267.
27. **Jaureguy, F., L. Landraud, V. Passet, L. Diancourt, E. Frapy, G. Guigon, E. Carbonnelle, O. Lortholary, O. Clermont, E. Denamur, B. Picard, X. Nassif, and S. Brisse.** 26 November 2008, posting date. Phylogenetic and genomic diversity of human bacteremic *Escherichia coli* strains. BMC Genomics **9:**560. [Epub ahead of print.]
28. **Kageyama, B., M. Nakae, S. Yagi, and T. Sonoyama.** 1992. *Pantoea punctata* sp. nov., *Pantoea citrea* sp. nov., and *Pantoea terrea* sp. nov. isolated from fruit and soil samples. Int. J. Syst. Bacteriol. **42:**203–210.
29. **Kidgell, C., U. Reichard, J. Wain, B. Linz, M. Torpdahl, G. Dougan, and M. Achtman.** 2002. *Salmonella typhi*, the causative agent of typhoid fever, is approximately 50,000 years old. Infect. Genet. Evol. **2:**39–45.
30. **Kratz, A., D. Greenberg, Y. Barki, E. Cohen, and M. Lifshitz.** 2003. *Pantoea agglomerans* as a cause of septic arthritis after palm tree thorn injury; case report and literature review. Arch. Dis. Child. **88:**542–544.
31. **Lim, P. S., S. L. Chen, C. Y. Tsai, and M. A. Pai.** 2006. *Pantoea* peritonitis in a patient receiving chronic ambulatory peritoneal dialysis. Nephrology (Carlton) **11:**97–99.
32. **Maiden, M. C.** 2006. Multilocus sequence typing of bacteria. Annu. Rev. Microbiol. **60:**561–588.
33. **Maiden, M. C., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtman, and B. G. Spratt.** 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. USA **95:**3140–3145.
34. **Maki, D. G., F. S. Rhame, D. C. Mackel, and J. V. Bennett.** 1976. Nationwide epidemic of septicemia caused by contaminated intravenous products. I. Epidemiologic and clinical features. Am. J. Med. **60:**471–485.
35. **Manulis, S., Y. Gafni, E. Clark, D. Zutra, Y. Ophir, and I. Barash.** 1991. Identification of a plasmid DNA probe for detection of strains of *Erwinia herbicola* pathogenic on *Gypsophila paniculata*. Phytopathology **81:**54–57.
36. **Matsaniotis, N. S., V. P. Syriopoulou, M. C. Theodoridou, K. G. Tzanetou, and G. I. Mostrou.** 1984. *Enterobacter* sepsis in infants and children due to contaminated intravenous fluids. Infect. Control **5:**471–477.
37. **Mergaert, J., L. Verdonck, and K. Kersters.** 1993. Transfer of *Erwinia ananas* (synomym, *Erwinia uredovora*) and *Erwinia stewartii* to the genus *Pantoea* emend. as *Pantoea ananas* (Serrano 1928) comb. nov., and *Pantoea stewartii* (Smith 1898) comb. nov., respectively, and description of *Pantoea stewartii* subsp. *indologenes* subsp. nov. Int. J. Syst. Bacteriol. **43:**162–173.
38. **Muytjens, H. L., and J. van der Ros-van de Repe.** 1986. Comparative in vitro susceptibilities of eight *Enterobacter* species, with special reference to *Enterobacter sakazakii*. Antimicrob. Agents Chemother. **29:**367–370.
39. **Naum, M., E. W. Brown, and R. J. Mason-Gamer.** 2008. Is 16S rDNA a reliable phylogenetic marker to characterize relationships below the family level in the *Enterobacteriaceae*? J. Mol. Evol. **66:**630–642.
40. **Olenginski, T. P., D. C. Bush, and T. M. Harrington.** 1991. Plant thorn synovitis: an uncommon cause of monoarthritis. Semin. Arthritis Rheum. **21:**40–46.
41. **Paauw, A., M. P. Caspers, F. H. Schuren, M. A. Leverstein-van Hall, A. Deletoile, R. C. Montijn, J. Verhoef, and A. C. Fluit.** 2008. Genomic diversity within the *Enterobacter cloacae* complex. PLoS ONE **3:**e3018.
42. **Posada, D.** 2006. ModelTest server: a Web-based tool for the statistical selection of models of nucleotide substitution online. Nucleic Acids Res. **34:**W700–W703.
43. **Rozas, J., J. C. Sanchez-DelBarrio, X. Messeguer, and R. Rozas.** 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics **19:**2496–2497.
44. **Salerno, A., A. Deletoile, M. Lefevre, I. Ciznar, K. Krovacek, P. Grimont, and S. Brisse.** 2007. Recombining population structure of *Plesiomonas shigelloides* (*Enterobacteriaceae*) revealed by multilocus sequence typing. J. Bacteriol. **189:**7808–7818.
45. **Smith, J. M., N. H. Smith, M. O'Rourke, and B. G. Spratt.** 1993. How clonal are bacteria? Proc. Natl. Acad. Sci. USA **90:**4384–4388.
46. **Spratt, B. G., W. P. Hanage, and E. J. Feil.** 2001. The relative contributions of recombination and point mutation to the diversification of bacterial clones. Curr. Opin. Microbiol. **4:**602–606.
47. **Spratt, B. G., and M. C. Maiden.** 1999. Bacterial population genetics, evolution and epidemiology. Philos. Trans. R. Soc. Lond. B **354:**701–710.
48. **van Belkum, A., P. T. Tassios, L. Dijkshoorn, S. Haeggman, B. Cookson, N. K. Fry, V. Fussing, J. Green, E. Feil, P. Gerner-Smidt, S. Brisse, and M. Struelens.** 2007. Guidelines for the validation and application of typing methods for use in bacterial epidemiology. Clin. Microbiol. Infect. **13**(Suppl 3)**:**1–46.
49. **Vincent, K., and R. M. Szabo.** 1988. *Enterobacter agglomerans* osteomyelitis of the hand from a rose thorn. A case report. Orthopedics **11:**465–467.
50. **Wayne, L., D. Brenner, R. Colwell, P. A. Grimont, O. Kandler, M. Krichevsky, L. Moore, W. Moore, R. Murray, E. Stackebrandt, M. 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.
51. **Weinthal, D. M., I. Barash, M. Panijel, L. Valinsky, V. Gaba, and S. Manulis-Sasson.** 2007. Distribution and replication of the pathogenicity plasmid pPATH in diverse populations of the gall-forming bacterium *Pantoea agglomerans*. Appl. Environ. Microbiol. **73:**7552–7561.
52. **Wirth, T., D. Falush, R. Lan, F. Colles, P. Mensa, L. H. Wieler, H. Karch, P. R. Reeves, M. C. Maiden, H. Ochman, and M. Achtman.** 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol. Microbiol. **60:**1136–1151.
53. **Young, J. M., and D. C. Park.** 2007. Relationships of plant pathogenic enterobacteria based on partial *atpD*, *carA*, and *recA* as individual and concatenated nucleotide and peptide sequences. Syst. Appl. Microbiol. **30:** 343–354.