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Systematic 16S rRNA Gene Sequencing of Atypical Clinical Isolates Identified 27 New Bacterial Species Associated with Humans

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Clinical microorganisms isolated during a 5-year study in our hospital that could not be identified by conventional criteria were studied by 16S rRNA gene sequence analysis. Each isolate yielded a ≥1,400-bp sequence containing <5 ambiguities which was compared with the GenBank 16S rRNA gene library; 1,404 such isolates were tested, and 120 were considered unique (27 isolates) or rare (≤10 cases reported in the literature) human pathogens. Eleven new species, "*Actinobaculum massiliae*," "*Candidatus* Actinobaculum timonae," *Paenibacillus sanguinis*, "*Candidatus* Bacteroides massiliae," *Chryseobacterium massiliae*, "*Candidatus* Chryseobacterium timonae," *Paenibacillus massiliensis*, "*Candidatus* Peptostreptococcus massiliae," "*Candidatus* Prevotella massiliensis," *Rhodobacter massiliensis*, and "*Candidatus* Veillonella atypica" were identified. Sixteen species were obtained from humans for the first time. Our results show the important role that 16S rRNA gene sequence-based bacterial identification currently plays in recognizing unusual and emerging bacterial diseases.

16S rRNA gene sequencing (39) was first proposed to identify uncultured microorganisms (17, 25, 38), but the idea that it could be used for bacterial identification of cultured bacteria in clinical laboratories has become increasingly realistic (7, 8, 34, 36), and the technique is now commercially available (6, 34, 35).

Identification of organisms by 16S rRNA gene sequencing has been described in case reports and in reports focusing on a particular bacterial phylum or genus, such as *Mycobacterium* (6, 36), *Actinomyces* (15, 28), aerobic gram-negative bacilli (8, 34), coryneform bacteria (35), and aerobic gram-positive rods (3), and we previously reported a series including both environmental and clinical isolates (7).

Although 16S rRNA gene sequence analysis has become a common method for the accurate identification of bacterial isolates (24), and its definition of bacterial species has been precise (30), the impact of systematic sequencing of unidentified bacteria in a clinical laboratory setting has seldom been evaluated (3). 16S rRNA gene sequence-based descriptions of new taxa are still a matter of debate (19). We therefore evaluated our experience in systematic sequencing of unidentifiable bacterial isolates for the purpose of describing new taxa and new clinical entities.

MATERIALS AND METHODS

Bacterial isolates. Our laboratory serves a 3,500-bed university-affiliated tertiary-care institution and routinely processes human samples for culture-based diagnosis of bacterial infectious diseases, including those due to anaerobes, aerobes, mycobacteria, and spirochetes. Infections by intracellular pathogens are diagnosed and researched in another laboratory and were not included in this study.

Our laboratory also acts as a regional center of expertise in microbial identification and receives unidentified bacterial isolates from all over the Marseilles metropolitan area. Conventional laboratory techniques used for phenotyping were carried out on all isolates according to standard methods and included Gram staining and manual (API strips; Bio Mérieux, La Balme-les-Grottes, France) or automated (Microscan; Dade Behring, Paris, France) determination of phenotypic characteristics for anaerobes and aerobes. For mycobacteria, the acid-fast status was determined, and phenotypic characteristics were determined manually, and 16S rRNA probe hybridization (GenProbe, San Diego, Calif.) was performed. Strict intracellular bacteria, bacterial isolates obtained during epidemiological investigations, and bacteria isolated from the environment were excluded from this study.

For all microorganisms obtained from clinical specimens, the criteria for 16S rRNA gene-based identification were as follows. The first was a probability of correct identification of <75% (Microscan) or <80% (API strip) regardless of the index of proximity to the most typical profile for each of the taxa. This value is given by the identification systems used in our laboratory. It estimates how closely the profile corresponds to the taxon relative to all the taxa in the database. In these systems, a correctly identified isolate has a probability of correct identification of >99%. This value is obtained after completion of phenotypic tests, that is, 12 to 48 h. The second was an antibiotic susceptibility pattern incompatible with phenotypic identification pattern. The third was identification of a *Corynebacterium* sp. or nontuberculous *Mycobacterium* sp. For each bacterial isolate, we obtained information on the nature of the submitted sample and clinical data for the patient, including age, gender, immune status, presence of indwelling devices, and site of the infection. Only one isolate per patient was further identified by 16S rRNA gene sequence analysis. Isolates representing new bacterial species (see below) were deposited in the Pasteur Institute Collection in Paris, France.

16S rDNA sequence analysis. Each isolate was streaked on chocolate agar or 5% sheep blood agar under the appropriate atmospheric and temperature conditions for growth. The purity of isolates was determined by macroscopic examination of colonies and microscopic examination of bacteria after Gram or Ziehl-Nielsen staining. Total DNA was extracted from one to five colonies, and amplification and sequencing of the 16S rRNA gene were performed as previously described (7). Searches of current nucleotide databases were carried out with the network service of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) with the BLAST algorithm. When the reference sequence in GenBank contained more than 1% ambiguities or gaps, we performed 16S rRNA gene sequencing of the corresponding type strain prior to determining the percentage of similarity. A >99% identity in 16S rRNA gene sequence was the criterion used to identify an isolate to the species level. A 97 to 99% identity in 16S rRNA gene sequence was the criterion used to identify an organism at the genus level, and <97% identity in 16S rRNA gene sequence was the criterion used to define a potentially new bacterial species (7). An isolate was defined as belonging to a new species when its phenotypic characteristics did not

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TABLE 1. Proportions of isolates which required 16S rRNA gene sequence analysis and numbers of unusual isolates

Bacteria	No. of isolates tested	No. (%) of isolates identified by 16S rRNA analysis	No. of rare or unique isolates[a]
Gram-positive cocci	75,537	300 (0.40)	6
Gram-positive rods[b]	16,487	524 (3.18)	86
Gram-negative bacteria			
Enteric bacteria	51,177	132 (0.26)	3
Other bacteria	26,357	225 (0.85)	14
Anaerobic bacteria	5,780	223 (3.86)	11
Total	175,338	1,404 (0.80)	120

[a] Isolates from human samples that have been reported 0 to 10 times.
[b] Including *Mycobacterium* spp.

match previously reported phenotypic profiles and its 16S rRNA gene sequence exhibited <97% similarity with the closest relative sequence.

Bibliographic search. To find the reported associations between bacterial species and the human infections they caused, we systematically searched for published cases with the names of the bacteria and the clinical samples from which they were isolated as key words. Key words for clinical samples included blood culture, urine, abscess, brain abscess, endocarditis, heart valve, and wound. First, a search was performed with the bibliography included in the *Manual of Clinical Microbiology* (22) dealing with the bacterial genus under study. If this produced fewer than 10 published cases, a second line search was performed with PubMed (http://www.ncbi.nlm.nih.gov), with the name of the bacteria and infection as key words. When published cases could still not be found, a third and final search was performed on GenBank (http://www.ncbi.nlm.nih.gov) with the name of the bacteria as the key word. When the nomenclature of the bacterial species had changed over the last 20 years, searches were performed with the various names used for the species, with either infection or the clinical samples from which isolates were obtained as key words. The bibliographic search was ended in January 2003. The isolates were classified as relatively common (>10 published cases), rare (≤10 published cases), or unique (no published cases associated with patients). We only recognized a published case of infection if it was in an indexed English-language journal, the bacteria had been identified precisely, there were demographic, epidemiological, and clinical data, and there was discussion of the potential role of the organism in the clinical features described.

RESULTS

During the study period, the laboratory tentatively identified a total of 175,338 isolates, of which 1,404 (0.8%) bacterial isolates could not be identified by conventional phenotypic criteria and were identified by 16S rRNA gene sequencing. For each isolate, a sequence of over 1,400 bp with fewer than five ambiguities was obtained and this enabled the organisms to be classified within bacterial groups, as presented in Table 1. One hundred twenty isolates belonged to rare bacterial species for which there were fewer than 10 published cases, i.e., 0.06% of our routine isolates. These isolates were obtained from blood ($n = 49$), aspirates from abscesses ($n = 14$), bone biopsies ($n = 18$), bronchopulmonary samples ($n = 9$), cutaneous biopsies ($n = 4$), joint fluid ($n = 5$), urine ($n = 4$), otorrhea ($n = 3$), heart valves ($n = 3$), stool samples ($n = 2$), peritoneal fluid ($n = 3$), wound swabs ($n = 2$), a lymph node ($n = 1$), a duodenal biopsy ($n = 1$), a nose ($n = 1$), and pleural fluid ($n = 1$). There was an overrepresentation of gram-positive rods among those subjected to 16S rRNA gene testing and the unusual isolates, since they represented 86 of 120 (71.6%) of the rare isolates.

Eleven isolates obtained from blood (eight isolates), urine (one isolate), a nose (one isolate), and a duodenal biopsy (one isolate) represented new species. They were preliminarily named "*Actinobaculum massiliae*" CIP 107404^T (12), "*Candidatus* Actinobaculum timonae," *Paenibacillus sanguinis* (27), "*Candidatus* Bacteroides massiliae," *Chryseobacterium massiliae* (11), "*Candidatus* Chryseobacterium timonae," *Paenibacillus massiliensis* (27), "*Candidatus* Peptostreptococcus massiliae," "*Candidatus* Prevotella massiliensis," *Rhodobacter massiliensis* (13), and "*Candidatus* Veillonella atypica" (Table 2). Sixteen isolates belonged to recognized species that have never been reported in humans and are regarded as environmental (Table 2). Microorganisms were recovered from blood in seven cases, bone biopsy in three cases, and a hip prosthesis, a cardiac valve, sputum, a abscess, peritoneal fluid, and a wound in one case each. Among these species, *Paenibacillus macerans* had previously been implicated in a pseudo-outbreak of bacteremia (23) and was finally regarded as an environmental contaminant. The list of 93 rare isolates is reported in Table 3.

DISCUSSION

Since the manual and automated techniques used in our laboratory are used universally for identifying bacteria (22), our results probably reflect the situation in most teaching hospital laboratories worldwide. In our experience, about 0.5 to 1% of the bacterial isolates recovered in our laboratory were not identified by phenotypic criteria and were submitted for molecular identification. Likewise, in their study of unidentifiable mycobacteria in a reference laboratory, Tortoli et al. estimated a prevalence of unknown mycobacteria slightly exceeding 1% (36). Bosshard et al. reported that 14% of all aerobic gram-positive rods isolated in their clinical microbiology laboratory would require 16S rRNA gene sequence-based identification (3). These data indicate that molecular identification of unidentifiable bacterial isolates offers an opportunity for the description of new bacterial species encountered in clinical microbiology laboratories. The fact that the commercially available 16S rRNA gene sequence-based identification kits have been favorably evaluated (34, 35) indicates that an exponential increase in the use of the technique can be expected within the next few years.

Indeed, 16S rRNA gene sequencing offers an unprecedented tool for the description of new bacterial species. Unlike a phenotypic test, it is a universal test because all bacterial species possess at least one 16S rRNA gene copy (39); also, it does not suffer from the variability observed for phenotypic tests between different observers, and it is easily transferable from one laboratory to another through electronic databases such as GenBank. In their study, Bosshard et al. reported 27 aerobic gram-positive rod isolates that appeared to be new species (3). However, the deposition of a 16S rRNA sequence in GenBank or similar databases is not enough to ensure a new species description, mainly because GenBank does not require the minimal basic data, such as source of isolation and normalized phenotypic criteria, as obligatory information. A minor difference or even a complete identity of the 16S rRNA gene sequence is not enough to assert that different isolates belong to the same species, but a reverse point of view is conceivable; if there are differences between two 16S rRNA gene sequences, the species are probably different.

TABLE 2. List of 27 bacterial isolates identified by 16S rRNA gene sequence analysis for the first time in humans

Species	Clade[a]	GenBank accession no.	Site of isolation	Patient information				Reference
				Age	Gender	Underlying condition	Clinical diagnosis	
Potential new species								
"*Actinobaculum massiliae*"	High-GC Gram+	AF487679	Urine	81 yr	F		Urinary tract infection	12
"*Candidatus* Actinobaculum timonae"	High-GC Gram+	AY008311	Duodenal biopsy		M		Enteritis catheter-related bacteremia	
Paenibacillus sanguinis	Low-GC Gram+	AY230764	Blood	52 yr	M	Rhinopharynx tumor	Bacteremia	27
"*Candidatus* Bacteroides massiliae"	*Bacteroides-Cytophaga*	AY126616	Blood	1 day	F	Prematurity	Premature rupture of membranes	
Chryseobacterium massiliae	Flavobacteria	AF531766	Nose		F	Homelessness	Rhinitis	11
"*Candidatus* Chryseobacterium timonae"	*Flavobacterium-Cytophaga*	AY244770	Blood	73 yr	M		Ulcerative colitis	
Paenibacillus massiliensis	Low-GC Gram+	AY230766	Blood	13 yr	M	Leukemia	Catheter-related bacteremia	27
"*Candidatus* Peptostreptococcus massiliae"	Low-GC Gram+	AY244772	Blood	44 yr	F	Burn	Sepsis	
"*Candidatus* Prevotella massiliensis"	*Bacteroides-Cytophaga*	AF487886	Blood	32 yr	M	Severe trauma	Bacteremia	
Rhodobacter massiliensis	α-Proteobacteria	AY244771	Blood		M	Mesothelioma	Bacteremia	13
"*Candidatus* Veillonella atypica"	Low-GC Gram+	AY244769	Blood	75 yr	F	Lung carcinoma	Bacteremia	
Species never isolated from humans before								
Arthrobacter nicotianae	High-GC Gram+	AJ315492	Hip prosthesis	40 yr	M	Hip prothesis	Sepsis	9
Clostridium glycolicum	Low-GC Gram+	AY244773	Peritoneal fluid	79 yr	F	Peritoneal neoplasm	Peritonitis	4
Corynebacterium coyleae	High-GC Gram+	AY244774	Blood	77 yr	M			10
Corynebacterium magusii	High-GC Gram+	AY244775	Wound	36 yr	M		Foot sepsis	
Desulfotomaculum guttoideum	Low-GC Gram+	Y11568	Bone	18 yr	M		Osteomyelitis	31
Haloanella gallinarum	*Bacteroides-Cytophaga*	AY244776	Bone		M		Osteomyelitis	21
Mesorhizobium amorphae	α-Proteobacteria	AY230775	Blood	3 yr	M	Cardiac abnormality surgery	Bacteremia	37
Microbacterium phyllosphaerae	High-GC Gram+	AJ277840	Blood	64 yr	M	Severe trauma	Bacteremia	1
Micromonospora floridensis	High-GC Gram+	X92621	Cardiac valve	33 yr	M	Bicuspid aortic valve	Endocarditis	20
Mycobacterium ratisbonense	High-GC Gram+	AF055331	Sputum	70 yr	M	Chronic bronchitis	Pneumonia	2
Nocardia corynebacteroides	High-GC Gram+	X80615	Abscess	40 yr	M	Hemodialysis	Intra-abdominal abscess	26
Paenibacillus amylolyticus	Low-GC Gram+	D85396	Blood	60 yr	F	Hemolytic neoplasm	Bacteremia	29
Paenibacillus macerans	Low-GC Gram+	AF273740	Bone	34 yr	M		Osteomyelitis	23
Paenibacillus stellifer	Low-GC Gram+	AY244777	Blood	52 yr	M	Aspiration pneumonia	Bacteremia	32
Streptomyces lydicus	High-GC Gram+	Y15507	Blood	74 yr	F	Chronic obstructive lung disease	Bacteremia	14
Streptomyces sampsonii	High-GC Gram+	D63871	Blood	45 yr	F	Thrombopenia	Bacteremia	33

[a] Gram+, gram positive.

We previously suggested that a 16S rRNA gene sequence similarity of <97% indicates that bacterial isolates belong to two different species (7). The same threshold was used in a recent study of aerobic gram-positive rods (3). However, a 16S rRNA gene sequence similarity of <3% does not always indicate that two isolates belong to the same species. The first isolate of any such new species must be deposited in a bacterial collection to allow comparisons. Currently, the only way to describe a new species is to publish in a peer-reviewed, international journal devoted to such research.

The minimum number of isolates required for the description of a new species is still a matter of debate; recent proposals for a minimum of five strains have been made (17, 19). Our data suggest that emerging species are encountered at a maximum rate of one every 4 years, so that application of a five-isolate rule would require 20 years before a new species could be described in a single place, a condition that would certainly result in significant underreporting of emerging bacterial species. Alternatively, our study suggests that any isolate with a previously undescribed phenotypic profile and a nearly complete 16S rRNA gene sequence that exhibits <97% similarity should be reported, deposited in GenBank, and deposited in an international collection. Accumulation of reports on the deposited species will allow further definition of its clinical significance. For example, the publication of our previous report on unusual bacterial isolates (7) brought 12 requests for strains over 2 years from laboratories worldwide which had isolated and characterized similar isolates. The formal description of a new species resulted from this collaborative effort after our initial report (18).

Alternatively, an electronic database devoted to bacteria of medical interest, such as the differentiation of medical microorganisms database (RIDOM) (16), in connection with international bacterial collections for the repository of isolates, may

TABLE 3. List of 93 isolates identified by 16S rRNA gene sequence analysis belonging to bacterial species reported ≤10 times as occurring in patients with the clinical diseases listed

Bacteria (no. of isolates)	Species (no. of isolates)	Clinical disease(s) (no. of cases)
Gram-positive cocci (n = 6)	*Abiotrophia defectiva*	Urinary tract infection
	Aerococcus christensenii	Bacteremia
	Enterococcus hirae	Osteitis
	Facklamia languida	Bacteremia
	Gemella bergeriae	Osteitis
	Helcococcus kunzii	Osteitis
Gram-positive rods (n = 69)	*Actinomyces gerencseriae* (2)	Osteitis, abscess
	Actinomyces graevenitzii (5)	Pneumonia (5)
	Actinomyces neuii (4)	Bacteremia (3), osteitis
	Actinomyces radingae (8)	Abscess (6), osteitis (2)
	Arcanobacterium bernardii (4)	Abscess (1), cutaneous lesion (1), urinary tract infection (1), wound (1)
	Arthrobacter cumminsii	Bacteremia
	Aureobacterium resistens	Cutaneous lesion
	Corynebacterium afermentans	Bacteremia
	Corynebacterium aquaticum (2)	Bacteremia (2)
	Corynebacterium aurimucosum (2)	Osteitis (2)
	Corynebacterium diphtheriae	Endocarditis
	Corynebacterium freneyi	Bacteremia
	Corynebacterium propinquum	Osteitis
	Corynebacterium pseudodiphterithicum (2)	Osteitis (2)
	Corynebacterium riegelii	Bacteremia
	Corynebacterium simulans	Osteitis
	Dermabacter hominis (11)	Abscess (3), arthritis (4), bacteremia (3), endocarditis (1)
	Desulfovibrio fairfieldensis	Bacteremia
	Gordonia terrae	Peritonitis
	Lactobacillus casei	Bacteremia
	Microbacterium lacticum	Bacteremia
	Microbacterium oxydans	Peritonitis
	Mycobacterium alvei	Pneumonia
	Mycobacterium bohemicum	Pneumonia
	Mycobacterium haemophilum	Osteitis
	Nocardia cyriacigeorgici	Abscess
	Nocardia otitidiscavarium (4)	Bacteremia (3), cerebral abscess (1)
	Nocardia transvalensis	Cutaneous lesion
	Nocardiopsis dassonvillei	Adenitis
	Oerskovia xanthineolytica	Bacteremia
	Rhodobacter timonae	Bacteremia
	Rothia mucilaginosa	Bacteremia
	Turicella otitidis (3)	Otorrhea (3)
Gram-negative bacteria		
Enteric bacteria (n = 3)	*Citrobacter amalonaticus*	Colitis
	Enterobacter asburiae	Urinary tract infection
	Salmonella give	Diarrhea
Other bacteria (n = 9)	*Acinetobacter schindleri*	Bacteremia
	Aeromonas jandaei	Bacteremia
	Halomonas venusta	Bacteremia
	Moraxella phenylpyruvica	Cutaneous lesion
	Ochrobactrum intermedium	Bacteremia
	Oligella urethralis (3)	Bacteremia (2), osteitis (1)
	Shewanella alga	Pneumonia
Anaerobic bacteria (n = 6)	*Clostridium scindens*	Bacteremia
	Eubacterium saburreum	Bacteremia
	Peptoniphilus harei (2)	Bacteremia (2)
	Prevotella buccae	Bacteremia
	Prevotella heparinolytica	Pleural effusion

encourage rapid proposals for new bacterial species by microbiological teams prior to formal description based on a series of several isolates. Also, a yearly published report on unusual bacterial isolates by microbiological laboratories involved in molecular identification of large numbers of isolates, such as ours, may help to collect basic data that allow further description of new species and emerging clinical entities.

Our study also demonstrates that 16S rRNA gene sequence analysis is a powerful technique to identify bacteria rarely associated with human infections, i.e., those reported fewer

than 10 times in the international literature, and thus contributing to the description of emerging diseases. In our study, such bacteria constituted 10% of the isolates that required further characterization by gene sequencing. Most of these unusual species were isolated from samples obtained from fine-needle aspirates or biopsies, and thus they were unlikely to be contaminants. Although isolation of a microorganism does not always indicate a causal relationship with disease, it does form the basis for the establishment of such a relationship. The unusual bacteria that we found were isolated from blood and aspirates from abscesses and bone, indicating bacteremia. We collected epidemiological, clinical, and phenotypic microbiological data in addition to 16S rRNA gene sequences for each of these unusual isolates, so that they may be published as case reports (11, 12, 13, 27). Indeed, such isolates fulfill recently proposed guidelines for publication of single-case reports (19).

Also, systematic description of any new bacterial species recovered from patients contributes to the description of emerging infectious diseases. Indeed, it allows international efforts toward the collection of cases and description of new clinical entities. Eleven isolates with 16S rRNA gene sequences exhibiting less than 97% sequence identity with other known bacterial sequences that possess new phenotypic profiles are potentially new species, and five have already been reported (11, 12, 13, 27). 16S rRNA gene sequence analysis allowed the determination of phylogenetic relationships among these isolates that would not have resulted from phenotypic data only. Although only one isolate was available in our laboratory for these new species, the availability of epidemiological, clinical, and phenotypic data along with <97% similar 16S rRNA gene sequence may allow the proposal of these isolates as prototypes (11, 12, 13, 27).

In summary, our 60-month study enabled us to find 11 new species that had less than 97% sequence identity with any known bacteria. Also, we identified 16 isolates belonging to species not previously recovered from patients and a total of 120 isolates that belonged to species which had been the subject of fewer than 10 clinical case reports. Altogether, our results show that up to 10% of the isolates selected for 16S rRNA gene sequence-based identification can be expected to be associated with extremely rare (≤10 reported cases) or undescribed infectious diseases. This may reach 20% for gram-positive rods and mycobacteria. Altogether, 0.06% of our routine isolates were identified as extremely rare or unique. Our study thus highlights the need for clinical microbiology laboratories to have molecular methods to supplement routine phenotypic identification methods for bacterial isolates.

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REFERENCES

1. **Behrendt, U., A. Ulrich, and P. Schumann.** 2001. Description of *Microbacterium foliorum* sp. nov. and *Microbacterium phyllosphaerae* sp. nov., isolated from the phyllosphere of grasses and the surface litter after mulching the sward, and reclassification of *Aureobacterium resistens* (Funke et al. 1998) as *Microbacterium resistens* comb. nov. Int. J. Syst. Evol. Microbiol. **51:**1267–1276.
2. **Berekaa, M. M., and A. Steinbuchel.** 2000. Microbial degradation of the multiply branched alkane 2,6,10,15,19,23-hexamethyltetracosane (squalane) by *Mycobacterium fortuitum* and *Mycobacterium ratisbonense.* Appl. Environ. Microbiol. **66:**4462–4467.
3. **Bosshard, P. P., S. Abels, R. Zbinden, E. C. Böttger, and M. Altwegg.** 2003. Ribosomal DNA sequencing for identification of aerobic gram-positive rods in the clinical laboratory (an 18-month evaluation). J. Clin. Microbiol. **41:**4134–4140.
4. **Chamkha, M., M. Labat, P. K. Patel, and J. L. Garcia.** 2001. Isolation of a cinnamic acid-metabolizing *Clostridium glycolyticum* strain from oil mill wastewaters and emendation of the species description. Int. J. Syst. Evol. Microbiol. **51:**2049–2054.
5. **Christensen, H., M. Bisgaard, X. Frederiksen, R. Mutters, P. Kuhnert, and J. E. Olsein.** 2001. Is characterization of a single isolate sufficient for valid publication of a new genus or species? Proposal to modify Recommandation 30b of the Bacteriological Code (1990 Revision). Int. J. Syst. Evol. Microbiol. **51:**2221–2225.
6. **Cloud, J. L., H. Neal, R. Rosenberry, C. Y. Turenne, M. Jama, D. R. Hillyard, and K. C. Carroll.** 2002. Identification of *Mycobacterium* spp. by using a commercial 16S ribosomal DNA sequencing kit and additional sequencing libraries. J. Clin. Microbiol. **40:**400–406.
7. **Drancourt, M., C. Bollet, A. Carlioz, R. Martelin, J. P. Gayral, and D. Raoult.** 2000. 16S ribosomal sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. J. Clin. Microbiol. **38:**3623–3630.
8. **Ferroni, A., I. Sermet-Gaudelus, E. Abachin, G. Quesne, G. Lenoir, P. Berche, and J. L. Gaillard.** 2002. Use of 16S rRNA gene sequencing for identification of nonfermenting gram-negative bacilli recovered from patients attending a single cystic fibrosis center. J. Clin. Microbiol. **40:**3793–3797.
9. **Funke, G., M. Pagano-Niederer, B. Sjoden, and E. Falsen.** 1998. Characteristics of *Arthrobacter cumminsii*, the most frequently encountered *Arthrobacter* species in human clinical specimens. J. Clin. Microbiol. **36:**1539–1543.
10. **Funke, G., C. P. Ramos, and M. D. Collins.** 1997. *Corynebacterium coyleae* sp. nov., isolated from human clinical specimens. Int. J. Syst. Bacteriol. **47:**92–96.
11. **Greub, G., B. La Scola, and D. Raoult.** Amoebae-resisting bacteria isolated from human nasal swabs by amoebal coculture. Emerg. Infect. Dis., in press.
12. **Greub, G., and D. Raoult.** 2002. "*Actinobaculum massiliae*," a new species causing chronic urinary tract infection. J. Clin. Microbiol. **40:**3938–3941.
13. **Greub, G., and D. Raoult.** 2003. Rhodobacter massiliensis sp. nov., a new amoebae-resistant species isolated from the nose of a patient. Res. Microbiol. **154:**631–635.
14. **Hain, T., N. Ward-Rainey, R. M. Kroppenstedt, E. Stackebrandt, and F. A. Rainey.** 1997. Discrimination of *Streptomyces albidoflavus* strains based on the size and number of 16S–23S ribosomal DNA intergenic spacers. Int. J. Syst. Bacteriol. **47:**202–206.
15. **Hall, V., P. R. Talbot, S. L. Stubbs, and B. I. Duerden.** 2001. Identification of clinical isolates of actinomyces species by amplified 16S ribosomal DNA restriction analysis. J. Clin. Microbiol. **39:**3555–3562.
16. **Harmsen, D., J. Rothagänger, M. Frosch, and J. Albert.** 2002. RIDOM: ribosomal differentiation of medical microorganisms database. Nucleic Acids Res. **30:**416–417.
17. **Hirschel, B., H. R. Chang, N. Mach, P. F. Piguet, J. Cox, J. D. Piguet, M. T. Silva, and L. Larsson.** 1990. Fatal infection with a novel, unidentified mycobacterium in a man with the acquired immunodeficiency syndrome. N. Engl. J. Med. **323:**109–113.
18. **Hoyles, L., E. Inganas, E. Falsen, M. Drancourt, N. Weiss, A. L. McCartney, and M. D. Collins.** 2002. Bifidobacterium scardovii sp. nov., from human sources. Int. J. Syst. Evol. Microbiol. **52:**995–999.
19. **Janda, J. M., and S. L. Abbott.** 2002. Bacterial identification for publication: when is enough enough? J. Clin. Microbiol. **40:**1887–1891.
20. **Koch, C., R. M. Kroppenstedt, and E. Stackebrandt.** 1996. Intragenetic relationships of the actinomycete genus *Micromonospora*. Int. J. Syst. Bacteriol. **46:**383–387.
21. **McBain, A. J., R. G. Bartolo, C. E. Catrenich, D. Charbonneau, R. G. Ledder, A. H. Rickard, S. A. Symmons, and P. Gilbert.** 2003. Microbial characterization of biofilms in domestic drains and the establishment of stable biofilm microcosms. Appl. Environ. Microbiol. **69:**177–185.
22. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken.** 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
23. **Noskin, G. A., T. Suriano, S. Collins, S. Sesler, and L. R. Peterson.** 2001. Paenibacillus macerans pseudobacteremia resulting from contaminated blood culture bottles in a neonatal intensive care unit. Am. J. Infect. Control **29:**126–129.
24. **Patel, J. B.** 2001. 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. Mol. Diagn. **6:**313–321.
25. **Relman, D. A., J. S. Loutit, T. M. Schmidt, S. Falkow, and L. S. Tompkins.** 1990. The agent of bacillary angiomatosis. An approach to the identification of uncultured pathogens. N. Engl. J. Med. **323:**1573–1580.
26. **Riess, F. G., and R. Benz.** 2000. Discovery of a novel channel-forming protein in the cell wall of the non-pathogenic *Nocardia corynebacteroides*. Biochim. Biophys. Acta **1509:**485–495.
27. **Roux, V., and D. Raoult.** Three novel *Paenibacillus* species from blood

cultures: *Paenibacillus timonensis* sp. nov., *Paenibacillus massiliensis* sp. nov., and *Paenibacillus sanguinis* sp. nov. Int. J. Syst. Evol. Microbiol., in press.
28. **Sabbe, L. J. M., D. Van De Merwe, L. Schouls, A. Bergmans, M. Vaneechoutte, and P. Vandamme.** 1999. Clinical spectrum of infections due to the newly described *Actinomyces* species *A. turicensis*, *A. radingae*, and *A. europaeus*. J. Clin. Microbiol. **37:**8–13.
29. **Shida, O., H. Takagi, K. Kadowaki, L. K. Nakamura, and K. Komagata.** 1997. Emended description of *Paenibacillus amylolyticus* and description of *Paenibacillus illinoisensis* sp. nov. and *Paenibacillus chibensis* sp. nov. Int. J. Syst. Bacteriol. **47:**299–306.
30. **Stackebrandt, E., W. Frederiksen, G. M. Garrity, P. A. D. Grimont, P. Kämpfer, M. C. J. Maiden, X. Nesme, R. Rossello-Mora, J. Swings, H. G. Trüper, L. Vauterin, A. C. Ward, and W. B. Whitman.** 2002. Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. Int. J. Syst. Evol. Microbiol. **52:**1043–1047.
31. **Stackebrandt, E., C. Sproer, F. A. Rainey, J. Burghardt, O. Pauker, and H. Hippe.** 1997. Phylogenetic analysis of the genus *Desulfotomaculum*: evidence for the misclassification of *Desulfotomaculum guttoideum* and description of *Desulfotomaculum orientis* as *Desulfosporosinus orientis* gen. nov., comb. nov. Int. J. Syst. Bacteriol. **47:**1134–1139.
32. **Suominen, I., C. Sproer, P. Kampfer, F. A. Rainey, K. Lounatmaa, and M. Salkinoja-Salonen.** 2003. Paenibacillus stellifer sp. nov., a cyclodextrin-producing species isolated from paperboard. Int. J. Syst. Evol. Microbiol. **53:** 1369–1374.
33. **Takeuchi, T., H. Sawada, F. Tanaka, and I. Matsuda.** 1996. Phylogenetic analysis of *Streptomyces* spp. causing potato scab based on 16S rRNA sequences. Int. J. Syst. Bacteriol. **46:**476–479.
34. **Tang, Y. W., N. M. Ellis, M. K. Hopkins, D. H. Smith, D. E. Dodge, and D. H. Persing.** 1998. Comparison of phenotypic and genotypic techniques for identification of unusual aerobic pathogenic gram-negative bacilli. J. Clin. Microbiol. **36:**3674–3679.
35. **Tang, Y. W., A. Von Graevenitz, M. G. Waddington, M. K. Hopkins, D. H. Smith, H. Li, C. P. Kolbert, S. O. Montgomery, and D. H. Persing.** 2000. Identification of coryneform bacterial isolates by ribosomal DNA sequence analysis. J. Clin. Microbiol. **38:**1676–1678.
36. **Tortoli, E., A. Bartoloni, E. C. Bottger, S. Emler, C. Garzelli, E. Magliano, A. Mantella, N. Rastogi, L. Rindi, C. Scarparo, and P. Urbano.** 2001. Burden of unidentifiable mycobacteria in a reference laboratory. J. Clin. Microbiol. **39:**4058–4065.
37. **Wang, E. T., M. A. Rogel, S. H. Sui, W. X. Chen, E. Martinez-Romero, and P. van Berkum.** 2002. *Mesorhizobium amorphae*, a rhizobial species that nodulates *Amorpha fruticosa*, is native to American soils. Arch. Microbiol. **178:**301–305.
38. **Wilson, K. H., R. Blitchington, R. Frothingham, and J. A. Wilson.** 1991. Phylogeny of the Whipple's-disease-associated bacterium. Lancet **338:**474–475.
39. **Woese, C. R.** 1987. Bacterial evolution. Microbiol. Rev. **51:**221–271.