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MINIREVIEW

Taxonomy and Identification of the *Burkholderia cepacia* Complex

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At the beginning of this review it is essential to clarify the terminology that will be used to refer to the members of the *Burkholderia cepacia* complex and their relatives. The name *B. cepacia* will relate only to *B. cepacia* genomovar I. Strains resembling *B. cepacia* may belong to the *B. cepacia* complex, to other *Burkholderia* species (for instance, *Burkholderia gladioli*), or to species from other genera (for instance, *Ralstonia pickettii*) that share some phenotypic or genotypic similarities with the *B. cepacia* complex. *B. cepacia* complex bacteria and organisms that may be confused with them will be altogether referred to as *B. cepacia*-like organisms. Most previous reports regarding these organisms were published before the recognition of the complicated taxonomic relationships between the different members of the *B. cepacia* complex; it is therefore unclear to what category the presumed *B. cepacia* isolates described would belong. For that reason, when such literature is cited, the name "*B. cepacia*" will be shown in double quotes.

Chronic microbial colonization of the major airways, leading to exacerbations of pulmonary infection, is the major cause of morbidity and mortality in patients with cystic fibrosis (CF). Typical CF pathogens include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae* (30). Other glucose nonfermenters, like *Stenotrophomonas maltophilia*, *Alcaligenes xylosoxidans*, *R. pickettii*, and *Burkholderia gladioli*, can frequently be found as well, but their role in the decline of pulmonary function is unclear (14, 19, 30). Several reports on the recovery of "*B. cepacia*" from CF patients appeared in the late 1970s and early 1980s (62, 63). The first detailed description of the clinical significance of "*B. cepacia*" colonization and infection was published in 1984 (47). In that seminal paper, Isles et al. documented the increasing prevalence of "*B. cepacia*" colonization and infection in the Toronto, Canada, CF treatment center and described the so-called "cepacia syndrome," a severe progressive respiratory failure with bacteremia that occurs in about 20% of all infected CF patients. Clustering of new cases in some centers and the decrease of colonization of new patients following segregation of colonized and noncolonized patients in other centers suggested that "*B. cepacia*" could be transmitted between CF patients. This was confirmed by several studies (34, 64, 67, 76, 84, 94) that showed that "*B. cepacia*" strains can spread between CF patients via simultaneous hospital admissions or social contact outside of the hospital. As a result of these findings, new guidelines were issued to reduce the risk of "*B. cepacia*" acquisition. These included discontinuing sponsorship and support of CF summer camps and segregation of colonized patients. Implementation of these draconian infection control measures has a tremendous impact on the lives of CF patients, and not all patients or caregivers accept such measures (35, 36, 62, 63).

"*B. cepacia*" can also cause lung infections in chronic granulomatous disease patients, and infections in these patients are associated with pneumonia and septicemia and are often lethal (2, 58, 72, 96). "*B. cepacia*" infections in immunocompetent patients occur only sporadically, but several cases of pseudoepidemics and nosocomial infections, often caused by contaminated disinfectants and anesthetic solutions, have been reported (3, 43, 50, 107).

Despite the advances that have been made in the understanding of the epidemiology, "*B. cepacia*" infections still have a considerable impact on morbidity and mortality in CF patients (18, 61, 62, 63). Since "*B. cepacia*" is resistant to most antimicrobial agents, effective therapies are not straightforward and management efforts are therefore aimed at prevention of infection (35, 63). Several recommendations regarding infection control measures have been made, and these include that CF patients should not share hospital rooms as inpatients and should limit contact in outpatient clinics (63). However, the efficiency of infection control measures are determined by the accuracy with which "*B. cepacia*" is diagnosed, and poor laboratory proficiency in identification of this organism still prevails (17, 40, 75). Although several guidelines intended to enhance accurate identification of bacterial species from sputum culture have been proposed by national CF organizations and by the International *Burkholderia cepacia* Working Group, the degree to which these are followed varies greatly among clinical microbiology laboratories (90).

The problem is given an extra dimension by the fact that several "*B. cepacia*" strains have attracted attention as antagonists of soilborne plant pathogens (44, 66) and as plant-growth-promoting agents that can colonize the rhizosphere of several economic crops and thereby increase the crop yield (9, 39, 74, 83). The exceptional metabolic diversity of this organism (which allows it to use, e.g., constituents of crude oils and herbicides as carbon sources) could be put to use in the bioremediation of recalcitrant xenobiotics (8, 28, 54, 57). However, most strains used or under development for biocontrol or

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TABLE 1. Overview of the genus *Burkholderia*[a]

Species name originally assigned[b]	*Burkholderia* species name or taxon assigned	Yr of assignment	Reference	Other name subsequently assigned	Yr of assignment	Reference
Pseudomonas cepacia	***B. cepacia* comb. nov. (*B. cepacia* genomovar I)**	1992	81, 104, 119			
Pseudomonas solanacearum	*B. solanacearum* comb. nov.	1992	119	*Ralstonia solanacearum* comb. nov.	1995	120
Pseudomonas pickettii	*B. picketti* comb. nov.	1992	119	*Ralstonia pickettii* comb. nov.	1995	120
Pseudomonas gladioli	*B. gladioli* comb. nov.	1992	119			
Pseudomonas mallei	*B. mallei* comb. nov.	1992	119			
Pseudomonas pseudomallei	*B. pseudomallei* comb. nov.	1992	119			
Pseudomonas caryophylli	*B. caryophylli* comb. nov.	1992	119			
Pseudomonas plantarii	*B. plantarii* comb. nov.	1994	102			
Pseudomonas glumae	*B. glumae* comb. nov.	1994	102			
	B. vandii sp. nov.	1994	102	Junior synonym of *B. plantarii*	1999	22
	***B. vietnamiensis* sp. nov. (*B. cepacia* genomovar V)**	1995	32, 104			
Pseudomonas cocovenenans	*B. cocovenenans* comb. nov.	1995	123	Junior synonym of *B. gladioli*	1999	22
Pseudomonas andropogonis	*B. andropogonis* comb. nov.	1995	32			
	***B. multivorans* sp. nov. (*B. cepacia* genomovar II)**	1997	104			
Pseudomonas glathei	*B. glathei* comb. nov.	1997	104			
Pseudomonas pyrrocinia	***B. pyrrocinia* comb. nov.**	1997	4, 104			
	B. thailandensis sp. nov.	1998	10			
	B. graminis sp. nov.	1998	109			
Pseudomonas phenazinium	*B. phenazinium* comb. nov.	1998	109			
	B. norimbergensis sp. nov.	1998	117	*Pandoraea norimbergensis* comb. nov.	2000	20
	B. caribensis sp. nov.	1999	1			
	***B. stabilis* sp. nov. (*B. cepacia* genomovar IV)**	2000	104, 105			
	B. kururiensis sp. nov.	2000	12			
	B. ubonensis sp. nov.	2000	118			
	B. fungorum sp. nov.	2001	23			
	B. caledonica sp. nov.	2001	23			
	***B. ambifaria* sp. nov. (*B. cepacia* genomovar VII)**	2001	25			
	***B. cepacia* genomovar III**	1997	104			
	***B. cepacia* genomovar VI**	2001	24			

[a] Members of the *B. cepacia* complex are in boldface type.

bioremediation purposes are taxonomically poorly characterized, and their potential hazard to the CF community is unclear (33, 37, 44, 110).

The taxonomic complexity of *B. cepacia*-like organisms and the lack of widespread and generally accepted identification schemes hinder sound studies that could establish the roles played by and the pathogenic significance of the different *B. cepacia*-like organisms. This information is crucial to propose scientifically founded policies for each of the above-mentioned problems. The purpose of this review is to present an overview both of the taxonomy of the *B. cepacia* complex and of the available phenotypic and genotypic methods aimed at the correct identification of these organisms.

TAXONOMY OF THE *B. CEPACIA* COMPLEX

Pseudomonas cepacia was originally described by Burkholder in 1950 as the causative agent of bacterial rot of onion bulbs (13). Other names that were assigned included eugonic oxidizers group 1, *Pseudomonas kingii*, and *Pseudomonas multivorans* (49, 77, 97), but several studies clearly showed that these could be considered as synonymous names of *P. cepacia* and that the name *P. cepacia* had priority (5, 86, 92, 95). The name *P. cepacia* was not included in the Approved List of Bacterial Names (93) and therefore lost standing in bacterial nomenclature until 1981, when it was revived by Palleroni and Holmes (81). In 1992, *P. cepacia* and six other species belonging to rRNA group II of the genus *Pseudomonas* (*Pseudomonas solanacearum*, *Pseudomonas pickettii*, *Pseudomonas gladioli*, *Pseudomonas mallei*, *Pseudomonas pseudomallei*, and *Pseudomonas caryophylli*) (82) were transferred to the new genus *Burkholderia* (119). In contrast to the genus *Pseudomonas*, the genus *Burkholderia* belongs to the β-subdivision of the phylum *Proteobacteria* (53). Since the genus name was first assigned, the taxonomy of the genus *Burkholderia* has undergone considerable changes (Table 1), and the genus now includes 22 validly described species: *B. cepacia* (the type species), *Burkholderia caryophylli*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Burkholderia gladioli*, *Burkholderia plantarii*, *Burkholderia glumae*, *Burkholderia vietnamiensis*, *Burkholderia andropogonis*, *Burkholderia multivorans*, *Burkholderia glathei*, *Burkholderia pyrrocinia*, *Burkholderia thailandensis*, *Burkholderia graminis*, *Burkholderia phenazinium*, *Burkholderia caribensis*, *Burkholderia kururiensis*, *Burkholderia ubonensis*, *Burkholderia caledonica*, *Burkholderia fungorum*, *Burkholderia stabilis*, and *Burkholderia ambifaria* (1, 10, 20, 22, 23, 24, 25, 32, 102, 104, 105, 109, 117, 118, 119, 120, 122, 123). A phylogenetic tree based on 16S rRNA gene sequences, showing the positions of all the *Burkholderia* species and representatives of related genera, is shown in Fig. 1.

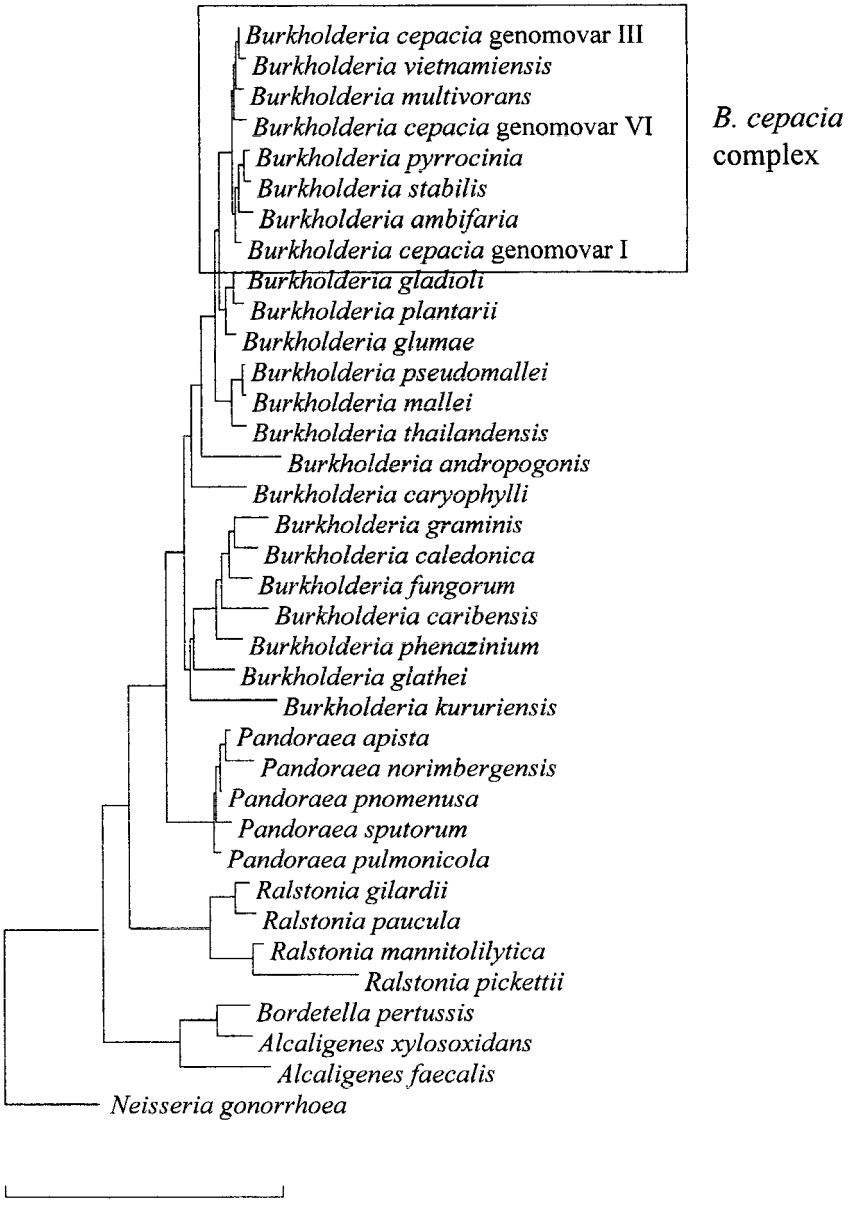

FIG. 1. Phylogenetic tree based on 16S rRNA gene sequences, showing the positions of all the *Burkholderia* species and of representatives of related genera. Bar, 10% sequence dissimilarity.

From the mid-1990s on, several researchers noted that there was a marked heterogeneity among "*B. cepacia*" strains isolated from different ecological niches. These strains were tentatively classified as "*B. cepacia*" using a wide range of techniques (7, 15, 32, 91, 100, 101, 121). The heterogeneity among "*B. cepacia*" isolates made correct identification problematic, and evaluation of the techniques used showed that they were either not very sensitive, not very specific, or neither sensitive nor specific (55, 59, 60, 69, 91, 98). The remarkable diversity among presumed "*B. cepacia*" strains and the lack of reliable identification schemes led Vandamme et al. (104) to a polyphasic taxonomic study that demonstrated that presumed "*B. cepacia*" strains isolated from CF patients and other sources belonged to at least five distinct genomic species or genomovars (the term genomovar was introduced to denote phenotypically similar genomic species [103]). *B. cepacia* genomovar V was identified as the previously described species *B. vietnamiensis* (32), and the name *B. multivorans* was proposed for the genomic species formerly known as *B. cepacia* genomovar II. The remaining groups were referred to as *B. cepacia* genomovars I, III, and IV. This group of five genomic species was collectively referred to as the *B. cepacia* complex. Since *B. cepacia* genomovar I contains the type strain, it retains the formal binomial name *B. cepacia*. Following a thorough investigation of the phenotypic and genotypic characteristics of *B. cepacia* genomovar IV strains (105), it became obvious that this organism could be differentiated from all other members of the *B. cepacia* complex, and it was formally classified as *B. stabilis*.

Subsequent polyphasic taxonomic studies identified two more members of the *B. cepacia* complex (24, 25). *B. cepacia* genomovar VI contains strains isolated from CF patients in the United States and the United Kingdom. This organism can phenotypically be differentiated from all members of the *B. cepacia* complex except *B. multivorans*. The name *B. ambifaria* (*B. cepacia* genomovar VII) was proposed for isolates from human clinical and environmental specimens, including CF patients. *B. ambifaria* also contains several well-characterized biocontrol strains. In addition, it was recently shown that the species *B. pyrrocinia* also belongs to the *B. cepacia* complex (4).

Within the *B. cepacia* complex, representatives of different species generally have DNA-DNA hybridization values between 30 and 60%, while values obtained from strains belonging to the same species are generally higher than 70%. DNA-DNA binding values obtained with other *Burkholderia* species are generally below 30% (22, 24, 25, 26, 32, 104). These values correspond to the three categories described in reference 106: high DNA relatedness (70% or higher) between strains of a single species, low but significant DNA relatedness below the species level, and nonsignificant DNA relatedness (30% or less). In addition, the similarities between 16S ribosomal DNA (rDNA) sequences obtained from different members of the *B. cepacia* complex are higher (>97.7%) than similarities between such sequences and those of other *Burkholderia* species (<97.0%) (Fig. 1).

IDENTIFICATION OF *B. CEPACIA* COMPLEX ORGANISMS

Introduction. The identification of organisms cultured from respiratory specimens obtained from CF patients is not straightforward. Using commercial systems, members of the *B. cepacia* complex have been misidentified as (among others) *B. gladioli*, *R. pickettii*, *Alcaligenes* spp., *Pseudomonas* spp., *S. maltophilia*, *Flavobacterium* spp., and *Chryseobacterium* spp., and strains of these various species have likewise been misidentified as belonging to the *B. cepacia* complex (55, 75). Methods for the identification of *B. cepacia*-like organisms must be capable of accurately identifying such a diverse variety of gram-negative nonfermenters, both distinguishing them from the *B. cepacia* complex and identifying the individual members of the *B. cepacia* complex. In addition, these methods should be relatively quick and easy to perform, given the clinical relevance of these organisms and the relatively large number of isolates involved (for example, the Cystic Fibrosis Foundation [CFF] *Burkholderia cepacia* Research Laboratory and Repository receives on average 750 *B. cepacia*-like isolates per year [J. J. LiPuma, Int. *Burkholderia cepacia* Working Group Abstr. 6th Annu. Meet., 2001 {Online}]).

Phenotypical tests. In routine clinical laboratories, the identification of putative *B. cepacia* complex isolates is generally performed using a combination of selective media, conventional biochemical analysis, and/or commercial systems (89, 108). Several different media have been developed for the selective isolation of *B. cepacia* complex isolates from sputum of CF patients. These media include *P. cepacia* medium (PC agar) (containing 300 U of polymyxin B per ml and 100 μg of ticarcilline per ml) (31); oxidation-fermentation agar supplemented with lactose, 300 U of polymyxin B per ml, and 0.2 U of bacitracin per ml (OFPBL agar) (113); and *B. cepacia* selective agar (BCSA) (containing 1% lactose and 1% sucrose in an enriched base of casein and yeast extract with 600 U of polymyxin B per ml, 10 μg of gentamicin per ml, and 2.5 μg of vancomycin per ml) (40). BCSA was reported to be superior to OFPBL and PCA in terms of rapidity (100% recovery following 72 h of incubation) and quality (70% of isolates showed good growth following 72 h of incubation) of recovery of *B. cepacia* complex organisms from CF respiratory specimens and inhibition of other organisms (41). Organisms not belonging to the *B. cepacia* complex that are capable of growth on BCSA include *B. gladioli* and *Ralstonia* spp. (41). The sensitivity and specificity of some or all of the above-mentioned media for the isolation of environmental "*B. cepacia*" isolates may be much lower (17), and therefore the use of other media, like PCAT medium (containing azelaic acid and tryptamine) (12) or TB-T medium (containing glucose, asparagine, trypan blue, and tetracycline) (38) may be recommended (4, 109).

There are several reports that describe the failure of most commercial test systems to identify *B. cepacia* complex isolates with sufficient sensitivity and specificity, with isolates commonly misidentified as *B. gladioli*, *S. maltophilia*, or *Ralstonia* spp. (55, 75, 89). Commercial test systems with relatively high positive predictive values (including the Vitek GNI Plus and Remel Uni-N/F Tek Plate and N/F Screen [89]) are available, but there is nevertheless a general consensus that bacterial isolates presumptively identified as belonging to the *B. cepacia* complex on the basis of commercial test system results should be tested for growth on BCSA, presence of lysine and ornithine decarboxylase activity, oxidation of sucrose and adonitol, presence of oxidase activity, hemolysis, pigment production, and growth at 42°C (42, 55, 75, 89, 108).

There are several phenotypic tests that allow the separation of *B. gladioli*, *Pandoraea* species, *R. pickettii*, *A. xylosoxidans*, and *S. maltophilia* from the *B. cepacia* complex (Table 2), and some of the members of the *B. cepacia* complex can be identified to the species or genomovar level based on phenotype. However, given the phenotypic variation that can occur within species and the frequent discrepancies between results obtained with different methodologies, the identification of *B. cepacia* complex based on phenotypic analysis alone should be confirmed by a reference laboratory equipped to provide more complete analyses (42). Consideration should also be given to the use of reference labs for any gram-negative nonfermenter for which species identification remains equivocal after phenotypic analysis.

Whole-cell protein analysis. Data presented by Vandamme et al. (104) indicated that sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of whole-cell proteins was a suitable technique for the identification of members of the *B. cepacia* complex. However, the comparison of the identification results obtained by this method with those obtained by other identification approaches revealed several discrepancies and a poor discrimination between *B. cepacia* genomovars I and III, *B. stabilis,* and *B. ambifaria* was noted. The advantages of this technique are its applicability to a wide range of organisms, the fact that little prior knowledge regarding the isolate is required, and its relative simplicity. A drawback of this method for the identification of *B. cepacia*-like isolates is that the whole-cell protein patterns are often characterized by

TABLE 2. Characteristics useful for the differentiation of members of the *B. cepacia* complex, *B. gladioli, Pandoraea* spp., *R. pickettii, A. xylosoxidans*, and *S. maltophilia*[a]

	Result for[b]:											
	B. cepacia							*B. gladioli*	*Pandoraea* species	*R. pickettii*	*A. xylosoxidans*	*S. maltophilia*
	Genomovar I	Genomovar II[c]	Genomovar III	Genomovar IV[d]	Genomovar V[e]	Genomovar VI	Genomovar VII[f]					
Oxidase	+	+	+	+	+	+	+	−	v	+	+	−
Oxidation of:												
Sucrose	v	−	v	−	+	−	+	−	−	−	−	v
Adonitol	v	+	v	v	−	+	+	+	−	−	−	−
Lactose	v	+	v	+	+	+	+	−	−	−	−	+
Lysine decarboxylase	+	v	+	+	+	−	+	−	−	−	−	+
Ornithine decarboxylase	v	−	v	+	−	−	−	−	−	−	−	−
Gelatine liquefaction	v	−	v	+	−	−	+	v	−	−	−	+
Aesculine hydrolysis	v	−	v	−	−	−	v	v	−	v	−	+
β-Galactosidase activity	+	+	+	−	+	+	+	+	−	−	−	+
Growth at 42°C	v	+	v	−	+	+	v	−	v	v	NK	v
β-Hemolysis	−	−	−	−	v	−	v	−	−	−	NK	NK

[a] Data for members of the *B. cepacia* complex, *B. gladioli*, and *R. pickettii* from reference 42; data for *Pandoraea* spp. from reference 20; data for *A. xylosoxidans* from references 52 and 111; and data for *S. maltophilia* from references 27, 80, and 111.

[b] +, 90% or more of the strains investigated yielded a positive reaction; −, 10% or fewer of the strains investigated yielded a positive reaction; v, between 10 and 90% of the strains investigated yielded a positive reaction; NK, not known. The numbers of strains investigated were 23 (*B. cepacia* genomovar I), 109 (*B. multivorans*), 139 (*B. cepacia* genomovar III), 27 (*B. stabilis*), 36 (*B. vietnamiensis*), 9 (*B. cepacia* genomovar VI), 18 (*B. ambifaria*), 27 (*B. gladioli*), 32 (*Pandoraea* spp.), and 12 (*R. pickettii*). The numbers of *A. xylosoxidans* and *S. maltophilia* strains investigated are not known.

[c] *B. multivorans.*

[d] *B. stabilis.*

[e] *B. vietnamiensis.*

[f] *B. ambifaria.*

a distortion of part of the banding pattern. These distortions significantly influence the correlation level between the protein patterns. Therefore, it is essential to compare the result of the numerical analysis of the protein patterns with the profiles themselves in order to delineate the clusters (21, 24, 25, 104). SDS-PAGE of whole-cell proteins remains a valuable tool for the identification of *B. cepacia* complex and *B. cepacia*-like isolates in the research setting, where experienced personnel are present for the interpretation of the protein profiles; the above-mentioned shortcomings, however, render it unsuitable for use in the clinical setting.

AFLP fingerprinting. In the past decade, various nucleic acid sequence-based methods have been developed for the identification and typing of bacterial pathogens (73, 79). One of these methods is amplified fragment length polymorphism (AFLP) fingerprinting, a fingerprinting technique based on the selective PCR amplification of genomic restriction fragments. This method combines broad applicability with high reproducibility and discriminatory power (45, 48, 85, 87). Other data (20, 24, 25, 26) indicate that AFLP fingerprinting is a technique that can be used for the identification of members of the *B. cepacia* complex and other *B. cepacia*-like bacteria. However, the method is technically demanding and labor-intensive and radioactive formats are impractical for clinical use (79). Significant progress has been made with the fluorescent format (26, 29, 56), but the high setup costs associated with the purchase of a DNA sequencer may be prohibitive for most laboratories. The high reproducibility of the banding patterns for a given strain facilitates database construction and use of such a database for identifying new bacterial strains. However, the presence of high-intensity bands in the patterns of some strains and the intermediate taxonomic position of several strains (as revealed by DNA-DNA hybridization) may ultimately require additional testing before some strains can be conclusively identified, again making this method unsuitable for application in routine diagnostic microbiology laboratories. It is, however, a valuable tool in taxonomic studies and a welcome addition to SDS-PAGE of whole-cell proteins for the identification of organisms easily misidentified by the latter method.

Whole-cell fatty acid analysis. The high degree of automation, the relative simplicity, and the fairly low costs associated with whole-cell fatty acid analysis make it a valuable technique for rapid identification of isolates in clinical laboratories (112). However, Vandamme et al. (104) reported the failure of whole-cell fatty acid analysis to distinguish between the first five known species of the *B. cepacia* complex, and more-recent data (105) confirmed this conclusion. It was also shown that fatty acid analysis cannot differentiate members of the *B. cepacia* complex from *B. gladioli* (116; Clode, F. E., A. Louise, L. Metherel, and T. L. Pitt, Letter, Am. J. Respir. Crit. Care Med. **160:**374–375, 1999; M. Wilsher, J. Kolbe, A. J. Morris, D. F. Welch, and P. A. R. Vandamme, Authors' Reply to Letter, Am. J. Resp. Crit. Care Med. **160:**374–375, 1999). From the comparison of published data, it is obvious that there are qualitative and quantitative differences in the fatty acid composition of members of the *B. cepacia* complex and other *B. cepacia*-like species, like *Pandoraea* spp. (20) and *Ralstonia* spp. (21), but considering standard deviations, it seems questionable whether these differences will suffice to identify all new isolates to the species level. Therefore, all organisms identified by whole-cell fatty acid analysis as belonging to the *B. cepacia* complex, *B. gladioli*, the genus *Pandoraea*, or the genus *Ralstonia* should be further investigated with methods more suitable for identification of *B. cepacia*-like isolates to the species level. A main advantage of this technique is the existence of a commercial database (Microbial ID) for identification of isolates that allows the rapid separation of *B. cepacia* complex organisms and related organisms both from other gram-negative nonfermenters (like *P. aeruginosa* and *S. maltophilia*) and from *Enterobacteriaceae*. The technique can also be used to assign isolates that cannot be classified with other screening methods to a major phylogenetic lineage.

PCR-based identification. Several candidate PCR assays aimed at the identification of "*B. cepacia*" have been described previously (16, 51, 78, 101) but most of these assays were developed before the recognition that the *B. cepacia* complex consists of several species. In addition, most relied on published DNA sequence data derived from analyses of culture collection strains that, in retrospect, are poorly representative of the total diversity within the *B. cepacia* complex. Most of the studies regarding PCR-based identification of members of the *B. cepacia* complex that have been carried out so far have been based on the diversity within the nucleotide sequences of the 16S and/or 23S rDNAs and were either aimed at the development of species- and/or genomovar-specific primers or RFLP analysis of the PCR-amplified 16S rRNA gene (6, 11, 24, 25, 65, 70, 88, 114, 115). The results from these studies clearly indicate that *B. multivorans*, *B. vietnamiensis*, and *B. cepacia* genomovar VI each can be separated from all other members of the *B. cepacia* complex. *B. cepacia* genomovars I and III, *B. stabilis*, *B. ambifaria*, and *B. pyrrocinia* can be identified as a group, but the variation within the rRNA operon is obviously too small to separate all members of the *B. cepacia* complex, and because of this discriminatory limitation, Mahenthiralingam et al. (70) developed a novel PCR-based identification assay based on the *recA* gene. The *recA* gene shows 94 to 95% similarity between the different genomovars, and typically 98 to 99% similarity can be found within the genomovars. However, *B. cepacia* genomovar I and III each contain two subpopulations with a different *recA* allele. At the moment of this writing, *recA* gene-derived primer pairs are available for the identification of *B. cepacia* genomovar I, *B. cepacia* genomovar III, *B. multivorans*, *B. stabilis*, *B. vietnamiensis*, and *B. ambifaria* (no primers are available yet for *B. pyrrocinia* or *B. cepacia* genomovar VI) (25, 70). In addition to *recA* gene-derived species-specific primers, a *recA* gene-based RFLP approach, enabling the recognition of multiple types within each genomovar, was developed (70).

The development of these novel molecular tools has provided the scientific community with quick, easy, and scientifically sound ways of identifying individual strains belonging to this taxonomically complex group of organisms. The disadvantages of the PCR-based methods include the need for appropriate measures to avoid cross-contamination (including the use of negative controls and the use of different areas for PCR manipulations) and the fact that PCR primers are not available for all *B. cepacia*-like organisms (e.g., no published primers are available yet for the identification of *Pandoraea* or *Ralstonia* species). In addition, care should be taken in the interpretation of negative PCR results (i.e., in distinguishing between true-

and false-negative results), and in general it can be stated that laboratories engaging in PCR-based identification of *B. cepacia* complex organisms should be appropriately equipped at the technical level and should comply with stringent quality control requirements to exclude misidentifications (46).

***B. cepacia* experimental strain panel.** Recently, a panel of 30 well-characterized strains representative of *B. cepacia* genomovars I and III, *B. stabilis*, *B. multivorans*, and *B. vietnamiensis* was assembled (71). The main reason for the assembly of this panel was that identification, epidemiological, and virulence studies all would benefit from the use of a defined set of representative strains. Since the assembly of the panel, several new taxa belonging to the *B. cepacia* complex have been described, and representative strains of these new taxa will have to be included in an updated version of the experimental strain panel.

CONCLUSIONS

It can be concluded that most of the methods necessary to identify *B. cepacia*-like organisms are available. The choice of what identification tools to use depends on their availability and the mission of the laboratory involved. In the research laboratory, a polyphasic approach (aimed at the integration of different kinds of data and information) (106) seems appropriate. Firstly, isolates should be assigned to a major phylogenetic group (such as the *B. cepacia* complex or the genus *Pandoraea*) using SDS-PAGE, whole-cell fatty acid analysis, or 16S rDNA sequence analysis. In addition, members of the *B. cepacia* complex that cannot unequivocally be identified to the genomovar level should be included in complementary screening methods like RFLP fingerprinting of the *recA* gene and/or AFLP fingerprinting. The identity of strains can then be confirmed using *recA* gene-based PCR assays or 16S rDNA RFLP fingerprinting. The mission and therefore the challenge posed by the identification of *B. cepacia*-like organisms for routine clinical microbiology laboratories is different. Strains isolated on selective media and tentatively identified as belonging to the *B. cepacia* complex using commercial systems should be confirmed with the classical biochemical tests described. The present state of the art indicates that isolates that are considered to be putative members of the *B. cepacia* complex after additional testing should be further examined by the genotypic methods discussed above. Laboratories equipped to augment routine evaluation with genotypic analyses have been established (e.g., the CFF *Burkholderia cepacia* Research Laboratory and Repository, as well as the Canadian *Burkholderia cepacia* Research and Referral Repository [for more information, please see the website http://go.to/cepacia]). The development of additional PCR-based identification systems and their wider use will have an important impact on studies that seek to elucidate the epidemiology and natural history of human infections due to *B. cepacia*-like organisms.

The early detection of *B. cepacia* complex and *B. cepacia*-like bacteria is extremely important both for the CF patient as well as for the CF community. However, a recent study (90) indicated that less than half of U.S. centers surveyed employ "*B. cepacia*"-specific selective media or incubate cultures for extended periods, both of which improve the yield of this organism. The use of these up-to-date culture techniques is technically not demanding and should be the expected standard of care in every CF center worldwide. Continuing education with regard to this issue is crucial. Apart from detection, correct identification of *B. cepacia*-like bacteria is extremely important. Therefore, priority should be given to the continuous evaluation of existing PCR-based methods (and other methods used for identification) with a view to keeping them up-to-date with respect to the increasing biodiversity found within the *B. cepacia* complex. It will also be useful to develop alternative PCR-based identification assays and expand the existing assays to related taxa like *R. pickettii* and *Pandoraea* spp. The use of up-to-date laboratory techniques for the proper detection and identification of *B. cepacia* complex organisms in respiratory cultures of CF patients will be beneficial to patients and CF centers, enhance the accuracy of national CF registries, and provide the basis for further studies. The improved diagnosis of infections caused by members of the *B. cepacia* complex and other *B. cepacia*-like organisms will help with the interpretation of the results from clinical outcome studies, and by doing so, will provide crucial information regarding the pathogenicity and/or transmissibility of specific strains involved.

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