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Identification of IS*1356*, a New Insertion Sequence, and Its Association with IS*402* in Epidemic Strains of *Burkholderia cepacia* Infecting Cystic Fibrosis Patients

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Burkholderia cepacia **is now recognized as an important opportunistic pathogen in cystic fibrosis (CF) and other compromised patients. Epidemicity among CF patients has been attributed to at least one particularly infectious strain (strain ET12), and both genetic evidence and anecdotal evidence suggest that this strain, currently endemic in Ontario, and those causing an epidemic in the United Kingdom, are indeed the same. Our study was conducted to determine whether there was any association between the presence of various insertion sequence (IS) elements, the cable pilin subunit gene (*cblA*), electrophoretic type (ET), and ribotype (RT) in a collection of 97 clinical and 2 environmental isolates of *B. cepacia*. No apparent linkage was found for IS elements IS*401*, IS*402*, IS*406*, IS*407*, and IS*408* with ET or RT. The *cblA* target, said to be a marker for high infectivity, was detected in 100% (38 of 38) of strains of *B. cepacia* ET12 and in a single strain of ET13 that differed in a single enzyme allele. A new IS, IS*1356*, identified during the investigation, was present in 71.7% of all isolates, and 50.7% of these isolates harbored IS*1356* as a hybrid IS element inserted into IS*402*. IS*1356* is 1,353 bp in length, and when it is inserted into IS*402* it results in a 10-bp duplication at the site of insertion. IS*1356* contains one major open reading frame of 1,260 bp coding for a putative transposase which has significant homology to IS*Rm3* in *Rhizobium meliloti* (59%) and to an undesignated IS element in *Corynebacterium diphtheriae* (49%). The IS*402*-IS*1356* element was found exclusively in the epidemic strains from Ontario and the United Kingdom, being detected in 94.7% (36 of 38 isolates) of *B. cepacia* ET12 isolates. Of the two ET12 isolates found to be devoid of the IS*402*-IS*1356* element, both contained IS*1356* unassociated with IS*402*, one was temporally unrelated to the epidemic, and the other was from a CF patient in a geographic area remote from Ontario and the United Kingdom. It is evident that the IS*402*-IS*1356* hybrid element, the *cblA* pilin subunit gene, and the allelic suite represented by multilocus enzyme electrophoretic type ET12 may provide useful markers for the epidemic, highly transmissible transatlantic strain isolated in Ontario and the United Kingdom.**

Burkholderia cepacia is an aerobic gram-negative bacillus commonly found throughout the environment and as a phytopathogen causing soft rot in onions (1). Over the past decade, however, strains which cause opportunistic infections in humans, most notably in cystic fibrosis (CF) patients, have been encountered with increasing frequency, leading to an increase in morbidity and mortality (12, 38). Among non-CF patients extrapulmonary nosocomial infections have more recently been reported (21).

Although the mechanism of virulence of *B. cepacia* isolates has not been elucidated (19), isolates from CF patients have been shown to adhere to mucin (26) and buccal epithelial cells (27). There may also be a correlation between the source of *B. cepacia* isolates (e.g., environmental and CF-associated epidemic and nonepidemic isolates) and the particular class of pili expressed (9). The implications of being colonized with *B. cepacia* isolates are a growing concern in the CF patient community, and markers of strain virulence are eagerly sought. Enhanced transmissibility and virulence appear to be strain dependent, and epidemic lineages are being defined anecdotally and genetically (10, 13, 17, 33–36). To date, studies have indicated cross-infection between patients (10, 17, 25, 31) and nosocomial acquisition (20) as important parameters of transmission.

In attempts to limit the spread of *B. cepacia* strains, many clinical centers now segregate colonized and noncolonized CF patients. This has proved to be successful but is limited by the social contacts between patients outside of the hospital setting that is the norm for CF patient groups, especially adults (10, 17, 31).

Many studies involving *B. cepacia* strains have focused on their truly extraordinary potential to metabolize a wide variety of organic compounds. It is currently thought that this metabolic versatility may, in part, be the result of the genomic complexity (24) and the large number of insertion sequence (IS) elements identified in *B. cepacia* strains (7, 15). IS elements have the ability to promote genomic rearrangement, recruit foreign genes, and cause insertional gene activation. Indeed, most of the IS elements in *B. cepacia* isolates have been identified by observing these features (16). The effect of IS elements on the genes with which they are associated is well documented (8), and it is conceivable that they may act genetically to increase the transmissibility and pathogenicity of certain strains of *B. cepacia.*

We originally identified the strains obtained by Govan et al. (10) in 1993 and ourselves (13, 25), from the United Kingdom

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TABLE 1. Oligonucleotide primers used to detect the various IS elements and *cblA* pilin subunit gene in *B. cepacia*

Target gene	Primer sequences	Amplicon size (bp)
IS*402*	A: 5′-CAA CCG AGA CTG AGG AGA TG-3′	
	B: 5′-GCT GCT TGC CAA TCG CGC TC-3′	250
IS*406*	A: 5′-GAC GGT GGG TCT GAC GCC AT-3′	
	B: 5′-AAG CCC TGA GTC CCT CGT CG-3′	450
IS*407*	A: 5′-TCA TCG GGT TTC TGA AGG AA-3′	
	B: 5′-CGG AAG CGA GCT GCA CGG TC-3′	750
IS*408*	A: 5′-TTG AAG GAA GTC CTG CGA CT-3′	
	B: 5′-TCG ACT TCG CCC AAT CCT TG-3′	370
IS*1356*	A: 5′-GGC CCT GAA GAA GGC GAT AT-3′	
	B: 5′-TCC GGC GAC ACC TCG ATG CC-3′	327
cblA[a]	A: 5′-CCA AAG GAC TAA CCC A-3′	
	B: 5′-ACG CGA TGT CCA TCA CA-3′	610

[a] Primers were first described by Sajjan et al. (28).

and Canada, respectively, as having an identical enzyme electrophoretic allotype (electrophoretic type 12 [ET12]), the first direct evidence that the anecdotal association of Canadian *B. cepacia* strains currently endemic in Ontario and those causing an epidemic in the United Kingdom were the same. In the present study, the frequencies of occurrence of various IS elements were studied in our collection of clinical *B. cepacia* strains to determine if any relationship exists between these genetic modifiers and electrophoretic type (ET) or ribotype (RT). Additionally, a recent publication by Sun et al. (36) described the presence of novel cable pili in the epidemic clone described above, and our collection of strains was therefore also screened for the presence of the *cblA* pilin subunit gene.

MATERIALS AND METHODS

Bacterial strains, nucleic acid preparation, ribotyping, and multilocus enzyme electrophoresis. The collection of strains used in the investigation consisted of 99 isolates of *B. cepacia*, most of which were previously characterized for their ETs and RTs (13). Strains were grown overnight on Columbia blood agar base (Quelabs, Montreal, Quebec, Canada) at 37°C in 5% CO_2 prior to nucleic acid (NA) extraction. The isolates were originally obtained as isolates either from CF patients or from nosocomial outbreaks, and they were maintained in the culture collection of the Laboratory Centre for Disease Control, Ottawa, Ontario, Canada. Two of the strains investigated were American Type Culture Collection (ATCC) reference strains of environmental origin (ATCC 17759 and ATCC 25416). Procedures for NA purification, ribotyping, and multilocus enzyme electrophoresis were as described previously (13).

Oligonucleotide primers and PCR amplification. The sequences of the oligonucleotide primers designed to detect the various IS elements and the pilin subunit gene are summarized in Table 1 and are based on the published sequences for IS*402* (6), IS*406* and IS*407* (41), IS*408* (2), and *cblA* (36). Primers for the detection of IS*1356* were designed on the basis of the sequence data acquired in the present investigation. All primers were synthesized on a 392 DNA-RNA Synthesizer (Applied Biosystems, Foster City, Calif.) by using standard phosphoramidite chemistry. Amplification was performed in a PE9600 thermocycler (Perkin-Elmer Cetus, Foster City, Calif.), with PCR mixtures containing 0.2 mg of NA per ml, 200 mM deoxynucleoside triphosphate, 1 mM (each) primer in the pair, 50 U of *Taq* polymerase (Boehringer Mannheim, Laval, Quebec, Canada) per ml, and 1× reaction buffer supplied by the manufacturer. Thermocycling conditions consisted of an initial denaturation of 2 min at 94°C; this was followed by 30 cycles of 30 s at 94°C, 30 s at 60°C, and 30 s at 72°C. Following amplification, the samples were incubated at 72°C for 10 min and were then cooled to 4°C. Amplicons were detected by electrophoresis in 2% agarose and staining with ethidium bromide (29).

Vectorette PCR was performed as described previously (23) by using primer IS*1356*-A (Table 1) as the target primer. Vectorette libraries were constructed with NAs from strain LCDC 92-498 (ET12, RT20) for *Bam*HI, *Bcl*I, *Bgl*II, *Eco*RI, *Hin*dIII, *Nhe*I, *Sal*I, *Spe*I, and *Xba*I. This isolate is a member of the group of strains implicated in the spread of *B. cepacia* ET12 between the United Kingdom and Canada (10, 13, 31). Amplification was performed as described above by using a two-step thermocycling profile of 30 cycles of 94°C for 30 s and 72°C for 3 min. The reaction mixtures were analyzed on a 1% low-melting-point agarose gel, and the resulting amplicons were excised from the gel and purified with the Wizard PCR Prep Purification system (Promega, Madison, Wis.).

Cloning and sequencing of IS*402*-IS*1356*. A bacteriophage library was constructed from strain LCDC 92-498 by using the ZAP Express Cloning Kit (Stratagene, La Jolla, Calif.). The probe was prepared by amplifying NA from strain LCDC 92-498 with the primers IS*1356*-A and IS*1356*-B (Table 1) in the presence of digoxigenin-11-uridine-5′-triphosphate (DIG). PCR conditions were identical to those used to detect the IS; however, the deoxynucleoside triphosphates were substituted with DIG Labeling Mix (Boehringer Mannheim). Positive clones were identified with the DIG DNA Detection Kit (Boehringer Mannheim) according to the manufacturer's directions. After purification of the bacteriophage clones, the phagemids were excised as directed in the ZAP Express Kit and plasmid DNA was purified with the Quiawell Plus Plasmid purification system (Quiagen, Chatsworth, Calif.) as recommended by the manufacturer.

Sequencing was performed on an ABI 373 automated DNA sequencer by using the Prism Dye Terminator sequencing kit (Applied Biosystems). Sequencing primers were designed on the basis of the acquired data as required to complete the sequence. Sequence analysis was performed with the various programs supplied with PG/Gene (Intelligenetics, Mountain View, Calif.) and Lasergene (DNAStar, Madison, Wis.). Phylogenetic analysis was performed with PAUP, version 3.0 (37).

IS designation and nucleotide sequence accession number. The IS*1356* designation was from Esther M. Lederberg (Stanford University School of Medicine, Stanford, Calif.), under the auspices of the Plasmid Reference Centre Prefix Registry.

The IS*402*-IS*1356* sequence has been assigned GenBank accession number U44828.

RESULTS

Frequency of targeted IS elements in *B. cepacia*. The frequencies of occurrence of targeted IS elements in *B. cepacia* isolates from environmental, nosocomial, and CF patient sources are recorded in Table 2. Overall, IS*402* was found in 68.7% of the isolates, IS*406* was found in 22.2% of the isolates, IS*407* was found in 48.5% of the isolates, IS*408* was found in 53.5% of the isolates, and IS*1356* was found in 71.7% of the isolates. In addition to these IS elements, primer sets were also designed to amplify IS*401* (2), but no amplicons were detected in this collection of isolates (data not shown). There were no apparent linkages between the presence of these IS elements and an ET or an RT, with one notable exception. The primers targeting IS*402*, in addition to detecting the IS element, also primed an additional amplicon of approximately 650 bp in some isolates. This anomaly was restricted to strains designated ET12, which is the ET of isolates documented to be highly transmissible in CF patients (10, 13, 31). Sequence data revealed that the 650-bp amplicon consisted initially of the

TABLE 2. Distribution of IS elements in *B. cepacia* isolates

Source	No. of isolates	No. (%) of isolates with the indicated IS element					
		IS*402*	IS*406*	IS*407*	IS*408*	IS*1356*	IS*402*-IS*1356*
Environmental	2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)
Nosocomial	15	10 (66.7)	7 (46.7)	2 (13.3)	12 (80.0)	4 (26.7)	0 (0.0)
CF patients	82	58 (70.7)	15 (18.3)	46 (56.1)	41 (50.0)	66 (80.5)	36 (43.9)
Total	99	68 (68.7)	22 (22.2)	48 (48.5)	53 (53.5)	71 (71.7)	36 (36.4)

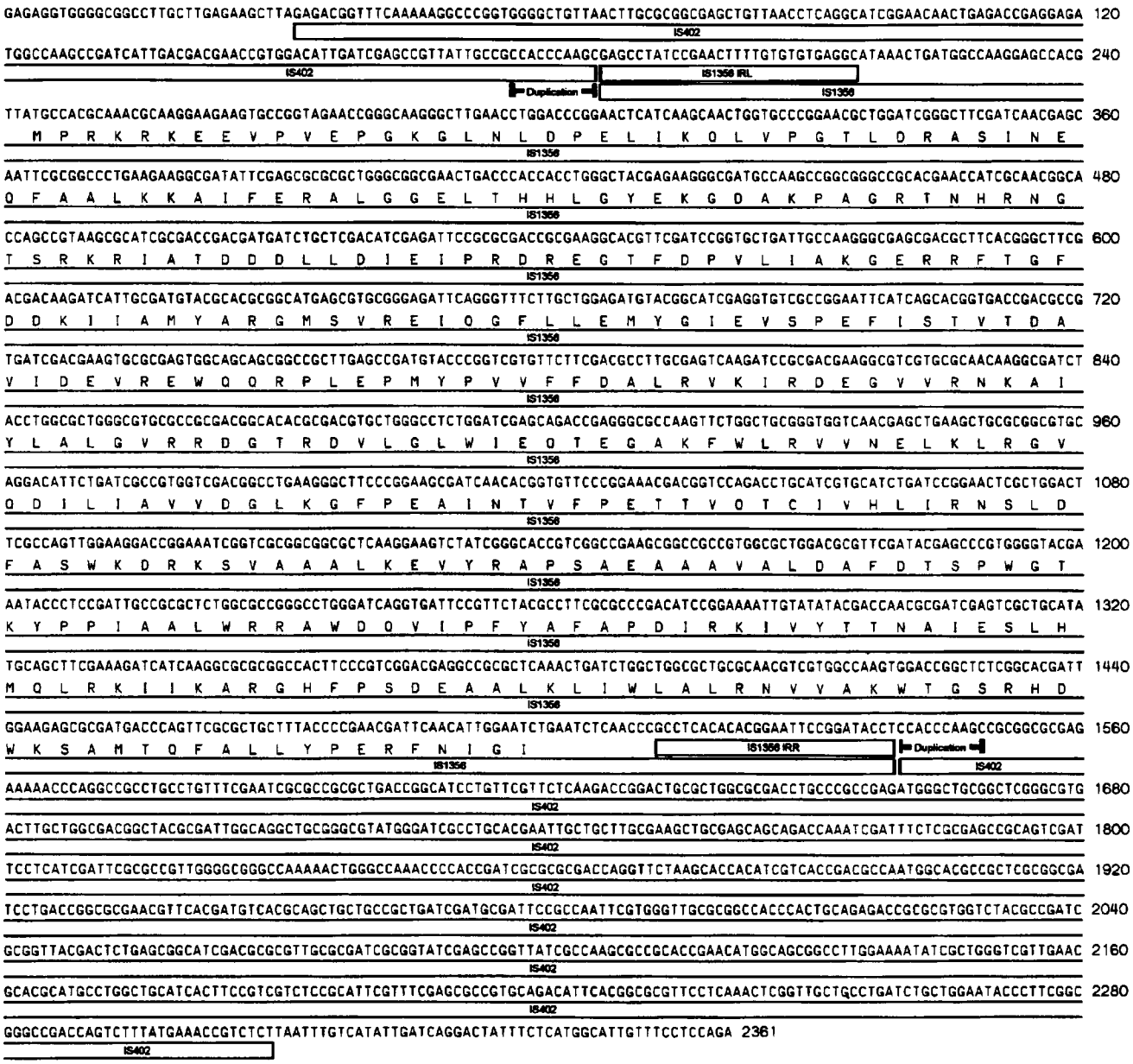

FIG. 1. Nucleotide sequence of IS*402*-IS*1356* and the amino acid sequence of the putative transposase of IS*1356*. The pertinent features of the IS*402*-IS*1356* element are illustrated below the nucleotide sequence. The locations of IS*402* and IS*1356* and their terminal inverted repeats are shown by the open boxes. The 10-bp duplication of IS*402* due to the insertion of IS*1356* is indicated by the solid bars.

IS*402* sequence, but this was interrupted after 154 bp and was succeeded by the sequence of IS*1356.*

Cloning and characterization of IS*402*-IS*1356*. Through the use of the vectorette PCR, an amplicon of approximately 1,300 bp was obtained from the *Bcl*I library and was used for sequencing. The *Bgl*I and *Eco*RI libraries also produced amplicons, but these were considerably smaller in size and were not investigated further. After sequencing of the amplicon, further attempts at "gene walking" through the use of vectorette PCR proved unsuccessful because of the large number of unresolvable amplicons obtained. From the bacteriophage library 10 candidate clones were selected for sequencing and allowed for the identification of three different insertion sites. Insertion into IS*402* is shown in Fig. 1. The two other sites identified were 5′-CTGACCGGCGG-IS*1356*-CCACCGGTGA-3′ and 5′-CGTTGTCTCG-IS*1356*-3′. The clone containing the latter insertion site did not contain the full IS*1356* sequence, and therefore, the 3′ insertion sequence is not known.

The IS*402*-IS*1356* element consists of the IS*402* reported by Ferrante and Lessie (6), including the 3-base duplication (5′-TTA-3′) at the insertion site. Although certain sequence differences were detected between the IS*402* sequence reported previously and the one present in the hybrid, these difference were not considered significant. The IS*402* sequence is interrupted after 154 bases by IS*1356*, resulting in a 10-bp duplication at the insertion site. IS*1356* is 1,353 bp in length and terminates at either end by imperfect inverted repeats. The left-hand inverted repeat (IRL) is 27 bp in length and the

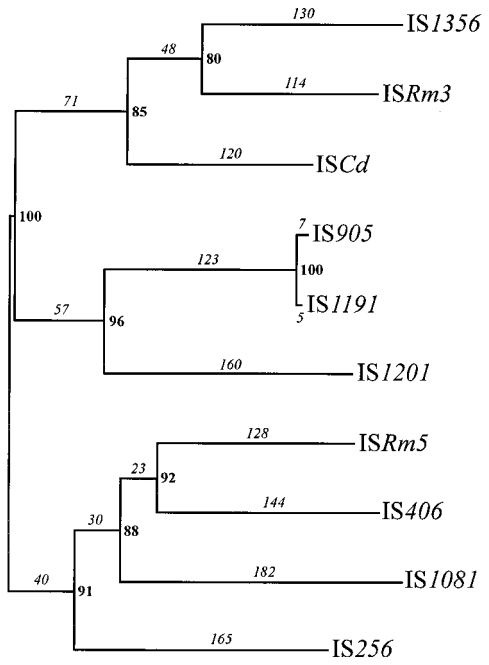

FIG. 2. Phylogenetic relationships among the putative transposases of various IS elements and that of IS*1356*. The dendrogram was generated after 100 bootstrap replications of branch-and-bound searches by using PAUP, version 3.0. The numbers along the branches indicate the branch lengths, and the numbers at the branch nodes are the respective bootstrap values. The transposases compared with that of IS*1356* were those found on IS*Rm3* from *R. meliloti* (40), IS*Cd* from *Corynebacterium diphtheriae* (22) (IS*Cd* is an unofficial designation for this element), IS*905* from *Lactococcus lactis* (5), IS*1191* from *Streptococcus thermophilus* (11), IS*1201* from *Lactobacillus helveticus* (39), IS*Rm5* from *R. meliloti* (14), IS*406* from *B. cepacia* (41), IS*1081* from *Mycobacterium bovis* (4), and IS*256* from *Staphylococcus aureus* (3).

right-hand inverted repeat (IRR) is 29 bp in length, with seven mismatches over the common region. IS*1356* contains one major open reading frame of 1,260 bp which codes for a putative transposase. This transposase showed significant homologies to several others found in the Swiss-Prot 31 database, with the most significant homologies of 59% to IS*Rm3* found in *Rhizobium meliloti* (40) and 49% to an undesignated IS found in *Corynebacterium diphtheriae* (22). The phylogenetic relationships between several of the more closely related transposases are shown in Fig. 2.

In order to determine if the IS*402*-IS*1356* element had a conserved insertion site, primers which would amplify either the 5′ or 3′ insertion sites were designed, and the resulting amplicons were sequenced (data not shown). All isolates in which the IS*402*-IS*1356* hybrid IS element was detected yielded an amplicon of the predicted size with a sequence identical to that originally identified.

Distribution of targeted IS elements and *cblA* pilin subunit genes among several *B. cepacia* ETs. Table 3 summarizes the distributions of the IS elements and the *cblA* pilin subunit genes in our collection of 99 strains representative of 20 ETs. When IS*1356* was detected in strains, the primers did not permit discrimination between IS elements found as the hybrid and IS*1356* located at other sites. The initial identification of the IS*402*-IS*1356* element was accomplished by observing the 650-bp band obtained with the IS*402* primer set; however, in these strains successful amplification of the target was difficult to reproduce, so the presence of the hybrid was confirmed by screening all isolates with the IS*402*-A and IS*1356*-B primers to obtain a 592-bp amplicon (data not shown).

Two of the ET12 isolates studied were found to lack the IS*402*-IS*1356* element. One of these isolates was found to possess all of the other IS elements investigated except IS*406*, and the other possessed IS*408* and IS*1356*. Neither of these two ET12 isolates lacking the hybrid element were clearly associated with the epidemic, in that one is a reference strain used many years ago to establish the serotyping scheme for *B. cepacia* (18) and the other came from a CF patient resident in

TABLE 3. Distribution of targeted genes in *B. cepacia* strains representing 20 ETs

ET	No. of isolates tested	Source[a]	No. of isolates with the indicated IS or gene						
			IS*402*	IS*406*	IS*407*	IS*408*	IS*1356*	IS*402*-IS*1356*	*cblA*
1	1	CF	1	0	1	1	1	0	0
2	1	CF	1	0	1	0	0	0	0
3	1	NS	1	0	0	1	0	0	0
4	2	CF	0	0	0	0	1	0	0
5	1	CF	0	0	1	0	0	0	0
6	3	CF	0	0	0	0	2	0	0
7	1	CF	0	0	0	0	0	0	0
8	2	NS	2	1	2	1	0	0	0
9	1	ENV	0	0	0	0	0	0	0
10	1	NS	0	0	0	1	0	0	0
11	5	NS	1	3	0	4	2	0	0
12[b]	38	CF	37	10	37	32	38	36	38
13[c]	1	CF	1	0	1	0	1	0	1
14	1	CF	0	0	1	0	1	0	0
15	1	NS	1	0	0	1	1	0	0
16	18	CF	9	2	2	1	18	0	0
17	12	CF	10	2	2	7	5	0	0
18	4	NS	4	4	0	4	0	0	0
19	1	ENV	0	0	0	0	1	0	0
20	4	CF	0	0	0	0	0	0	0

[a] CF, CF patient; ENV, environmental; NS, nosocomial.
[b] Epidemic transatlantic clone (Canada and the United Kingdom).
[c] Isolate from a Canadian CF patient in a province remote from Ontario and with no known epidemic association.

a remote area of northern Ontario with no known association with areas of the provincial epidemic in the south. The *cblA* pilin subunit gene was found in all ET12 isolates tested and one strain of ET13 from an adult CF patient in a province remote from Ontario. There is no documented evidence of an epidemic association or the spread of this single ET13 isolate, which differs in only one esterase allele from ET12 and which is IS*402*-IS*1356* negative (Table 3). The *cblA* amplicon from this isolate was subjected to sequence analysis and was found to be identical to that in the ET12 isolates (data not shown).

DISCUSSION

In our earlier study (13), we demonstrated that while RTs appear to be highly variable in a geographical context, ETs seem relatively stable in a population of *B. cepacia* isolates from particular clinical sources and may, in fact, be the best indicator of a clonal distribution. Considering the large number of ISs know to reside in *B. cepacia* strains and their propensity to cause genetic rearrangements, the variability of RTs is not surprising. Since a number of IS elements found in *B. cepacia* isolates have been shown to affect the expression of associated genes in other circumstances (6, 16, 30, 41), we questioned if there were any linkages between carriage of particular IS elements, transmissibility, and/or virulence and ET type.

During the course of our investigations we succeeded in identifying a previously unreported IS element, which has been designated IS*1356*. This sequence has a structure typical of ISs in that it is terminated by inverted repeats, contains an open reading frame which spans virtually its entire length, and codes for a putative transposase. An interesting feature of IS*1356* is the high degree of similarity of the transposase to a variety of others identified on IS elements from a wide distribution of organisms. During the characterization of IS*Rm5*, Laberge et al. (14) observed that there appears to be a family of IS elements, of which IS*1356* is now a member, which share a common ancestry, even though the hosts of these IS elements are from highly divergent bacterial families. From an evolutionary standpoint these similarities are quite intriguing. Similarities between IS elements from *B. cepacia* and *R. meliloti* strains could possibly be explained by their close association with respect to environmental habitat, but the ancestral relationship between these organisms and the other members of this family is difficult to fathom. Another interesting feature of IS*1356* is its insertion into IS*402*. While this arrangement is not unprecedented (14, 32), it proved to be unique to a particular clonal cluster of *B. cepacia* (the ET12 cluster) and was not found in other isolates. In addition, the site of insertion of the IS*402*-IS*1356* element was identical in all of the isolates investigated, further supporting the clonal nature of this group of isolates.

Although a large number of IS elements have been identified in *B. cepacia* strains, our study of their distribution was limited by the fact that only a small number of these have been sequenced. For those for which sequence data were available, no relationship between ET, RT, and IS carriage was found with the exception of the association of the IS*402*-IS*1356* element with ET12. The IS*402*-IS*1356* element was only found in *B. cepacia* strains isolated from CF patients and was restricted to ET12, with 36 of 38 (94.7%) of these isolates harboring the hybrid. Two of the 38 ET12 isolates examined lacked the IS*402*-IS*1356* element, although they both contained IS*1356* alone. Neither strain could be directly linked to the epidemic clone, because one was an early isolate used to establish a serotyping scheme and therefore was temporally unrelated and the other was isolated from a CF patient living in a remote location in Ontario with no obvious connections to the urban epidemic in that province.

The current study did not directly address this issue; however, it was noted that there appeared to be a certain bias in the distribution of IS elements among the clinical isolates with respect to the source of the host from which the strains were isolated. The IS elements IS*406* and IS*408* appeared more frequently in nosocomial isolates, whereas the IS elements IS*407* and IS*1356* appeared more frequently in isolates from CF patients. IS*402* was equally distributed between the two groups, and the IS*402*-IS*1356* element was found exclusively in CF patients and was restricted to ET12 isolates. Admittedly, the number of nosocomial isolates investigated was low and a wider sampling may alter the apparent bias in IS element distribution; however, it is also possible that this bias may reflect an ancestral branching of *B. cepacia* strains with respect to their clinical significance and association with a particular disease manifestation.

At present it is not known if the IS*402*-IS*1356* element is linked to the apparent increase in transmissibility and virulence of *B. cepacia* ET12, only that there is an "association." It is quite possible that the hybrid IS is simply a marker for this particular lineage of *B. cepacia*. Although the present study was not designed to demonstrate a direct effect of IS elements on virulence, the possibility of this type of association is not precluded. The importance of IS elements in the adaptability of *B. cepacia* isolates should not be ignored, and while they may not be directly related to virulence, they may, nonetheless, play an important indirect role in pathogenicity by assisting in the acquisition of virulence factors in certain strains.

The pathogenicity of *B. cepacia* and the implications of colonization with this organism are of considerable interest to those involved in the management of CF patients. Anecdotal evidence has recently linked a highly transmissible ET12 clone currently endemic in Ontario, Canada, to that causing an epidemic in the United Kingdom (10, 13). Sun et al. (36) demonstrated that the infectious, transatlantic clone was characterized by both *cblA* gene sequences and a similar chromosomal fingerprint. In the current study we found that the *cblA* pilin subunit gene was present in 100% (38 isolates from 18 patients) of the epidemic ET12 isolates in our collection of *B. cepacia* strains. However, it was also found in one ET13 isolate from an adult CF patient living in a province which has never been particularly associated with epidemics of any nature. Since Sun et al. (36) also identified a single unrelated isolate with a divergent *cblA* NA sequence, the PCR amplicon from our ET13 isolate was sequenced and was found to be identical to that characterized for the epidemic strain. In view of this evidence it would appear that, upon a wider sampling, the *cblA* pilin subunit gene may prove to be more invariant among unrelated strains than reported by Sun et al. (36).

In addition to the *cblA* pilin subunit gene association, Sun et al. (36) also presented limited ribotyping data and phylogenetic analysis which appeared to support the clonal nature of these strains. In their investigation, two very similar ribotype patterns were identified for the epidemic strains, and it was suggested that this general profile could also be used to identify these strains. However, in our earlier work (13), in addition to the two RT patterns identified by Sun et al. (36), we identified five other RT patterns within the epidemic strains using an identical methodology. It should also be noted that the group of bands which were invariant among the ET12 isolates were also seen in other unrelated isolates of various ETs. With this in mind it would appear that the claims made by Sun et al. (36) as to the usefulness of ribotyping as a screening method were somewhat premature and perhaps overly optimistic.

In view of the potential impact on future protocols for the management of *B. cepacia*-colonized CF patients, caution is advised in the screening of isolates solely on the basis of markers attributed to a single highly transmissible strain. It is clear that the *cblA* pilin subunit gene, the ET12 genotype, and the IS*402*-IS*1356* element appear to correlate well with this one particular epidemic strain, and the ability to identify this clone is not without value. However, as Sun et al. (36) also acknowledge, other distinct and highly transmissible epidemic lineages for which no genetic marker is currently available appear to exist. If any of these methods were prematurely implemented to screen CF patients, we would risk placing patients in the wrong groups, and the consequences in the case of these CF patients would be clinically and personally tragic.

The genome of *B. cepacia* is exceedingly complex, having three large chromosomal elements and a large plasmid, an arrangement apparently unique among eubacteria (24), and more genes involved in metabolism and pathogenicity need to be identified in order to provide important information on the virulence of clinical isolates. In view of the current data, it would appear that several genetic loci may need to be characterized and to agree in order to accurately assign clinical isolates to epidemic lineages. Until more is known about the pathogenicity of *B. cepacia* and the resulting implications to the CF community, we recommend that caution be applied before establishing any definitive phenotypic or genotypic screening criteria.

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