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Distribution of Quorum-Sensing Genes in the *Burkholderia cepacia* Complex

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The distribution of quorum-sensing genes among strains from seven genomovars of the *Burkholderia cepacia* complex was examined by PCR. *cepR* and *cepI* were amplified from *B. cepacia* genomovars I and III, *B. stabilis*, and *B. vietnamiensis*. *cepR* was also amplified from *B. multivorans* and *B. cepacia* genomovar VI. *bviIR* were amplified from *B. vietnamiensis*. All genomovars produced *N*-octanoyl-L-homoserine lactone and *N*-hexanoyl-L-homoserine lactone. *B. vietnamiensis* and *B. cepacia* genomovar VII produced additional *N*-acyl-L-homoserine lactones.

Burkholderia cepacia is an opportunistic pathogen that infects patients with cystic fibrosis (CF) (10, 12, 13). Some patients infected with *B. cepacia* develop cepacia syndrome, a necrotizing, often fatal pneumonia sometimes associated with bacteremia (14). Colonization with *B. cepacia* correlates with an increased risk of mortality at all levels of pulmonary function (4). The transmissibility of *B. cepacia* between CF patients (11, 15, 20, 26) and intrinsic resistance to a wide variety of antibiotics (25) are of increasing concern in the CF community.

B. cepacia was originally classified in the genus *Pseudomonas* but was transferred to the genus *Burkholderia* in 1992 on the basis of rRNA sequence analysis (35). Recently, *B. cepacia* has been classified into genotypically distinct species or genomovars referred to as the "*B. cepacia* complex" (3, 31; Coeyne et al., submitted for publication). Genomovars are phenotypically similar but genotypically distinct groups of strains that show a low level of DNA hybridization. The *B. cepacia* complex currently includes seven genomovars referred to as *B. cepacia* genomovar I, *B. multivorans* (formerly genomovar II), *B. cepacia* genomovar III, *B. stabilis* (formerly genomovar IV), and *B. vietnamiensis* (also known as genomovar V) (3, 31, 32) and two newly identified genomovars, genomovars VI (3) and VII (Coeyne et al., submitted).

Currently it is not known if these *Burkholderia* species possess different virulence factors or regulate virulence factors differently and subsequently vary in their pathogenicity. Strains of the *B. cepacia* complex produce a number of potential virulence factors, including siderophores, proteases, lipase, hemolysins, and pili (reviewed in references 10, 12, and 13). Production of extracellular virulence factors does not likely correlate with specific genomovars, since the majority of *B. cepacia* complex isolates produce these factors. Three markers have been associated with transmissible isolates, including cable pili (28), which have been shown to mediate adherence to respiratory mucins (21); an open reading frame of unknown function with homology to transcriptional regulators, termed the *B. cepacia* epidemic strain marker (19); and a hybrid of insertion sequences IS*402* and IS*1356* (30). These three markers have been predominantly found in isolates of *B. cepacia* genomovar III (2).

Quorum sensing is a signaling mechanism used by bacteria for the coordinate regulation of genes (5, 9, 22, 34). Quorum sensing involves the production of autoinducer signaling molecules, which are normally *N*-acyl homoserine lactones (AHLs) in gram-negative bacteria, and a transcriptional regulator. Quorum sensing regulates virulence factors, motility, biofilm formation, plasmid transfer, and antibiotic resistance (5, 34).

We have previously described the *B. cepacia* CepIR quorum-sensing system that was identified in *B. cepacia* genomovar III strain K56-2 (16). The autoinducer synthase gene, *cepI*, directs the synthesis of *N*-octanoyl-L-homoserine lactone (OHL) and *N*-hexanoyl-L-homoserine lactone (HHL) (16, 17). The transcriptional regulator, CepR, has been shown to negatively regulate biosynthesis of the siderophore ornibactin and positively regulate protease, OHL, and HHL production (16, 17). A second autoinducer synthetase gene, *bviI*, was identified in *B. vietnamiensis* DBO1 using random Tn*Mod* mutagenesis (6). Quorum-sensing genes have also recently been described in another strain of *B. vietnamiensis* (B. Conway and E. P. Greenberg, Abstr. 5th Annu. Int. *Burkholderia cepacia* Working Group Meet., 2000, p. 17, http://www.go.to/cepacia). The objectives of the present study were to determine if the *cepIR* and *bviIR* genes were present in other genomovars of the *B. cepacia* complex and to determine the autoinducer profiles of representative strains in the *B. cepacia* complex.

The distribution of *cepIR* and *bviIR* was determined in representative strains of the *B. cepacia* complex by PCR (Table 1; Fig. 1). The oligonucleotide primers and PCR conditions used are listed in Table 2. Genomic DNA was isolated from cultures grown in Luria-Bertani (LB) broth (Life Technologies, Burlington, Ontario, Canada) as described by Ausubel et al. (1). *Taq* polymerase and oligonucleotide primers were purchased from Life Technologies. PCRs were carried out in 50-μl volumes with the following amounts of reagents: 3.2 pmol of primer, 250 ng of DNA, 2.5 U of Platinum Taq Polymerase, a

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TABLE 1. Presence of quorum-sensing genes in strains of the *B. cepacia* complex

Species and strain	Source (location) or genotype	*cepI*	*cepR*	*bviI*	*bviR*	*cepI* reporter bioassay	Reference or source
B. cepacia genomovar I							
ATCC 25416	Onion (United States)	+[a]	+	−[b]	−	+	18
ATCC 17759	Soil (Trinidad)	+	+	−	−	+	18
CEP509	CF (Australia)	+	+	−	−	+	18
B. multivorans							
C5393	CF (Canada)	−	+	−	−	+	18
249-2	Laboratory (United States)	−	+	−	−	+	18
LMG13010	CF (Belgium)	−	+	−	−	+	18
B. cepacia genomovar III							
J2315	CF (United Kingdom)	+	+	−	−	+	18
K56-2	CF (Canada)	+	+	−	−	+	18
C5424	CF (Canada)	+	+	−	−	+	18
K56-I2	*cepI*::tmp	+	+	−	−	+	16
K56-R2	*cepR*::Tn*5*-OT182	+	+	−	−	+	16
B. stabilis							
LMG14294	CF (Belgium)	+	+	−	−	+	18
LMG14291	CF (Belgium)	+	+	−	−	+	18
LMG07000	Blood (Sweden)	+	+	−	−	+	18
B. vietnamiensis							
PC259	CF (United States)	+	+	+	+	+	18
LMG15232	CF (Sweden)	+	+	+	+	+	18
LMG10929	Rice (Vietnam)	+	+	+	+	+	18
G4[c]	Environment	+	+	+	+	+	B. Conway
DBO1[c]	Environment	+	+	+	+	+	33
B. cepacia genomovar VI							
LMG18943	CF	−	+	−	−	+	3
LO6	CF	−	+	−	−	+	3
B. cepacia genomovar VII							
ATCC 53266	Soil (United States)	−	−	−	−	+	T. Coenye
CEP996	CF (Australia)	−	−	−	−	+	T. Coenye
AMMD	Biocontrol strain	−	−	−	−	+	T. Coenye

[a] +, presence of the gene or activity in the autoinducer bioassay.
[b] −, absence of the gene or the activity in the autoinducer bioassay.
[c] Typed as *B. vietnamiensis* by the Cystic Fibrosis Foundation *B. cepacia* research laboratory and repository (J. LiPuma, personal communication).

0.2 mM concentration of each deoxynucleotide triphosphate (Amerisham Pharmacia Biotech, Inc., Baie d'urfé, Quebec, Canada), 3 mM $MgCl_2$, 5 μl of 10× buffer, and 10 μl of Q solution (Qiagen, Mississauga, Ontario, Canada). PCR products were separated on 0.8% agarose gels in Tris-acetate buffer. The plasmid pSLA3.2 (16) containing the *cepIR* genes was used as a positive control template for *cepI* and *cepR* amplification. The plasmids p824-E-3, which contains *bviI*, and p823-E-9, which contains *bviR*, were used as positive controls for amplification of *bviI* and *bviR*, respectively. The plasmid pUCP28T (23) was used a negative control for all PCRs.

Two primer combinations were used to amplify *cepR*. An 866-bp amplicon containing the complete open reading frame of *cepR* was amplified using primer set one, CEPR1 and CEPR2, and a 494-bp product containing the N-terminal 163 of 239 amino acids of CepR was amplified with primer set two, CEPR1 and CEPR3. With the exception of strains CEP509 (genomovar I) and C5393 (genomovar III), primer set one amplified *cepR* in the genomovar I, *B. multivorans*, genomovar III, *B. stabilis*, and *B. vietnamiensis* strains examined (data not shown). *cepR* was not amplified from strains of either genomovar VI or VII using these primers (data not shown). The primers CEPR1 and CEPR3 amplified an approximately 500-bp product in all strains with the exception of the three genomovar VII strains (Fig. 1A; Table 1). CEPR1 in combination with CEPR4, which amplifies a 575-bp product, also resulted in a negative PCR with the three genomovar VII strains (data not shown).

A 598-bp fragment containing the N-terminal 173 of 202 amino acids of CepI was amplified with the primers CEPI1 and CEPI2 in strains of genomovars I and III, *B. stabilis*, and *B. vietnamiensis* but not in *B. multivorans* or genomovars VI and VII (Fig. 1B). CEPI1 and CEPI3, which amplify a 278-bp product containing the first 93 amino acids of CepI, amplified this product from the same strains (data not shown).

Amplicons of *cepI* and *cepR* from one strain from each genomovar were cloned into the Topo vector pCR 2.1 (Invitrogen, Carlsbad, Calif.), and the nucleotide sequences were determined with the ABI PRISM DyeDeoxy Termination Cycle Sequencing System using AmpliTaq DNA polymerase (Perkin-Elmer Corp.) and the M13 universal primers and primers internal to *cepIR*. Reactions were performed with the ABI1371A DNA sequencer at the University Core DNA Services (University of Calgary). Sequence alignments were performed using

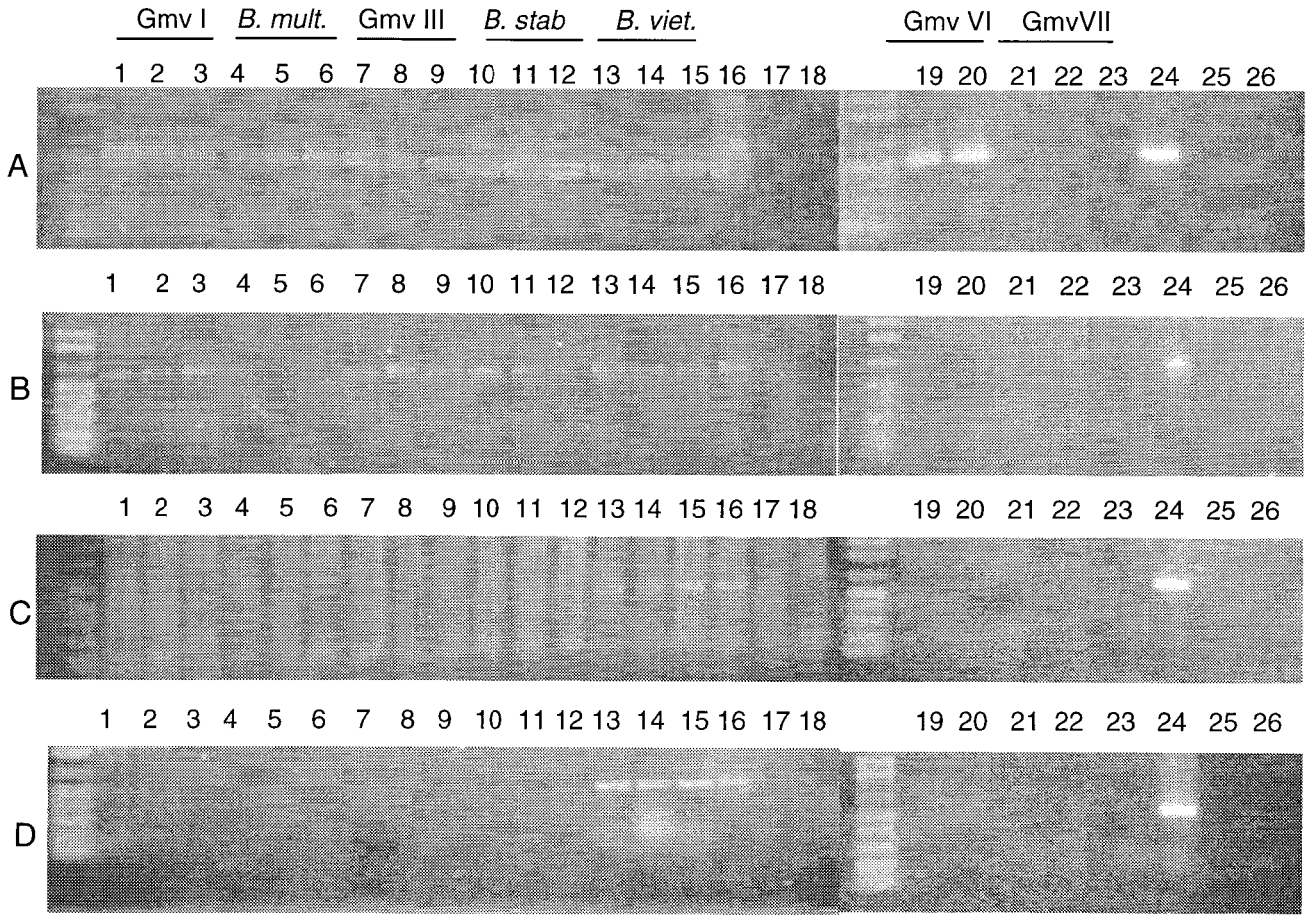

FIG. 1. Detection of *cepIR* and *bviIR* genes in strains of the *B. cepacia* complex by PCR. (A) *cepR*; (B) *cepI*; (C) *bviI*; (D) *bviR*. Lanes 1, ATCC 25416; lanes 2, ATCC 17759; lanes 3, CEP 509; lanes 4, C5393; lanes 5, 249-2; lanes 6, LMG13010; lanes 7, K56-2; lanes 8, J2315; lanes 9, C5424; lanes 10, LMG14294; lanes 11, LMG07000; lanes 12, LMG14291; lanes 13, PC259; lanes 14, LMG16232; lanes 15, LMG10929; lanes 17, pUCP28T; lanes 18, H_2O control; lanes 19, LMG18943; lanes 20, L06; lanes 21, ATCC 53266; lanes 22, AMMD; lanes 23, CEP996; lanes 25, pUCP28T; lanes 26, H_2O control. Lanes 16 and 24 contain the positive controls pSLA3.2 (A and B) p824E-9 (C), and p824E-3 (D). Abbreviations: Gmv, genomovar; *B. mult., B. multivorans; B. stab., B. stabilis; B. viet., B. vietnamiensis*.

DNAMAN Sequence Analysis Software (Lynnon Biosoft, Vandreuil, Quebec, Canada). The 866-bp *cepR* PCR product was sequenced, with the exception of genomovar VI LO6. In this instance the 494-bp amplicon containing only the first 163 amino acids of the predicted *cepR* open reading frame was cloned and sequenced. The predicted amino acid sequences were compared to those of genomovar III, strain K56-2 CepI and CepR (Table 3). The percent identity for CepR ranged from 99% in *B. vietnamiensis* PC259 to 92% in genomovar VI strain LO6. The percent identity for CepI ranged from 96% in *B. vietnamiensis* strain PC259 to 90% in *B. stabilis* strain LMG14291. These results indicate that *cepI* and *cepR* are highly conserved among the strains examined in most of the genomovars in the *B. cepacia* complex.

bviI was identified in *B. vietnamiensis* strain DBO1 as DBO6R using a random plasposon mutagenesis strategy, as previously described (6). Tn*Mod*-KmO was introduced from *Escherichia coli* DH5α into DBO1 by triparental mating with *E. coli* HB101 (pRK2013) (7). The DNA fragment containing the plasposon's site of insertion was cloned by performing a total genomic DNA digestion with *Pst*I (Life Technologies) Bethesda, Md. (a restriction enzyme that does not cut within the plasposon), purifying the digested products with GeneClean (Bio 101, Santa Clara, Calif). ligating with T4 DNA ligase (Life Technologies), electroporating into *E. coli* DH5α, and selecting on LB medium containing 50 μg of kanamycin per ml. The DNA sequence flanking the plasposon's site of insertion was determined using the primers JD45 (5′-ACGCT CAGTGGAACG-3′) and JD48 (5′-TTCCCGTTGAATATG GC-3′) and an ABI 377 DNA sequencer. The Tn*Mod*-KmO plasposon was inserted 301 bp from the start of the *bviI* open reading frame. The original cloned DNA fragment containing the rescued plasposon did not contain the complete sequence of the cognate response regulator *bviR*; therefore, the genomic DNA from *B. cepacia* complex strain DBO6R was digested with *Bam*HI in order to isolate a larger DNA fragment flanking Tn*Mod*-KmO. This fragment was similarly cloned as described above. The DNA sequence of *bviR* was obtained using a combination of primer walking and from *Eco*RI subclones constructed in the nested deletion vector p824 (J. J. Dennis, and G. L. Zylstra, submitted for publication).

bviI encodes a 219-amino-acid protein with 36% identity to CepI over the first 204 amino acids (Fig. 2). The *bviI* open reading frame encodes a product that is is 17 amino acids

TABLE 2. Primers and PCR conditions for amplification of quorum-sensing genes in *B. cepacia*

Gene	Primer	Sequence	Annealing temp (°C)[a]	Amplicon size (bp)
cepI	CEPI1	5′-GCGGATCC-$_{-121}$-ACCAGACGCCCATCTACCTGCTTCG-3′$_{134}$[b]	59	598
	CEPI2	$_{699}$5′-GTTACCAGTTACAGGCTCCTC-3′$_{679}$		
cepI	CEPI1	5′-GCGGATCC-$_{-121}$-ACCAGACGCCCATCTACCTGCTTCG-3′$_{134}$	59	278
	CEPI3	$_{390}$5′-GTATCTGCTGAACTCGCTGTTC-3′$_{379}$		
cepR	CEPR1	5′-CGGGATCC-$_{-1347}$-GAGAAAGAATGGAACTGCGC-3′$_{1366}$	55	866
	CEPR2	$_{2217}$5′-TCAGCAGAAGCTCGAGCAGAT-3′$_{2197}$		
cepR	CEPR1	5′-CGGGATCC-$_{-1347}$-GAGAAAGAATGGAACTGCGC-3′$_{1366}$	55	494
	CEPR3	$_{1845}$5′-ATGAAGCGGCTCAGCGAAT-3′$_{1824}$		
cepR	CEPR1	5′-CGGGATCC-$_{-1347}$-GAGAAAGAATGGAACTGCGC-3′$_{1366}$	55	575
	CEPR4	$_{1992}$5′-TTGTTCACGTGGAAGTTGAC-3′$_{1973}$		
bviI	BVII1	$_{1341}$5′-CGCAAAGTATCGGCATAAGG-3′$_{1322}$	55	600
	BVII2	$_{846}$5′-CTGTTCGTCGATCTCGATCCC-3′$_{866}$		
bviR	BVIR1	$_{3231}$5′-GGAATTTGACGGTGCGGTCG-3′$_{3212}$	55	471
	BVIR2	$_{2760}$5′-ATGCTGCAGTCCAACTATCC-3′$_{2779}$		

[a] PCR conditions were 94°C for 3 min (1 cycle) and 94°C for 1 min, annealing at the indicated temperature for 1 min, and 72°C for 1 min (30 cycles).
[b] Underlined region represents *Bam*HI site.

longer than CepI. The *bviR* open reading frame encodes a protein of 237 amino acids that is 36% identical to CepR (Fig. 2). The primers BVII1 and BVII2 amplified a 600-bp product internal to *bviI* only in strains of *B. vietnamiensis* (Fig. 1C). The primers BVIR1 and BVIR2 amplified a 471-bp product internal to *bviR* in all representative strains from *B. vietnamiensis* but not in strains from the other genomovars (Fig. 1D).

B. cepacia K56-I2, a *cepI* mutant, was used as a reporter strain to detect OHL production by each representative strain (16). When OHL is produced it binds CepR and restores protease production to K56-I2. Strains were streaked perpendicularly to the reporter strain grown on D-BHI (Becton Dickinson, Sparks, Md.)-milk agar (27), and protease production by the reporter was measured after incubation for 48 h. All of the strains tested were able to cross-feed the *cepI* reporter, suggesting that either OHL or other AHL molecules that can activate CepR are produced regardless of whether or not *cepI* or *bviI* was detectable by PCR using the indicated primers.

To determine the AHLs produced by strains of the various genomovars, an *Agrobacter tumefaciens* reporter previously shown to detect AHLs with 3-oxo-, 3-hydroxy-, and 3-unsubstituted side chains of all lengths, with the exception of *N*-butanoyl-L-HSL, was employed to examine AHL production in one strain of each genomovar. *A. tumefaciens* A136 does not contain a Ti plasmid coding for an autoinducer synthetase (36). This strain with plasmids pCF18, which harbors *traR*, and pCF372, with a *traI-lacZ* reporter, allows the detection of exogenous autoinducer production (8, 36). In the presence of AHLs, β-galactosidase activity observed from the *traI-lacZ* reporter is detected by a blue zone at the location of migration on thin-layer chromotography (TLC).

AHLs were extracted from 20-ml cultures grown in tryptic soy broth (Becton Dickinson) from one representative strain of each genomovar. Supernatants were extracted twice with equal volumes of acidified ethyl acetate (0.1 ml of glacial acetic acid per liter). Ethyl acetate was removed by rotary evaporation, and the residue was resuspended in 2 ml ethyl acetate, dried over N_2 gas, and resuspended in 100 μl of acidified ethyl acetate. TLC bioassays were performed as described elsewhere with modifications (24). Samples were spotted onto C_{18} reversed-phase TLC plates (20 by 20 cm; Whatman) and developed using methanol-water (60:40, vol/vol). The plates were overlaid with a *A. tumefaciens* A136 culture prepared as follows. A 3-ml overnight culture was diluted 1/100 into 30 ml of LB and grown to log phase. Cells were pelleted by centrifugation, resuspended in 20 ml of AT (29)–0.5% glucose medium, and incubated for 30 min. This culture was added to 150 ml of AT supplemented with 0.7% agar and 5-bromo-4chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (60 μg/ml). TLC plates were incubated for 24 h at 30°C. Synthetic *N*-hexanoyl-HSL, *N*-octanoyl-HSL and *N*-decanoyl-HSL (Fluka) were used as reference standards.

As previously reported (16, 17), *B. cepacia* K56-2 produces OHL and HHL. AHLs with R_f values corresponding to those of synthetic OHL and HHL were detected in extracts in all of the other strains examined (Fig. 3). In addition to OHL and HHL, *B. vietnamensis* also produced two other AHLs that may be *N*-decanoyl-HSL and *N*-dodecanoyl-HSL. Production of these four AHLs by *B. vietnamiensis* G4 has previously been reported (Conway and Greenberg, Abstr. 5th Annu. Int. *Burkholderia cepacia* Working Group Meet.). Since *bviI* was only amplified by PCR in *B. vietnamensis* it is likely that this gene is responsible for the production of one or both of these AHLs. The genomovar VII strain also produced another AHL that migrates between OHL and HHL on the TLC plate (Fig. 3).

These studies suggest that the *cepIR* genes are widely distributed in all genomovars of the *B. cepacia* complex and that *B. vietnamiensis* has at least two sets of quorum-sensing genes. In the strains examined, the *cepI* genes were shown to be highly

TABLE 3. Percent identity between predicted amino acid sequences of genomovar III CepIR and CepIR homologues in other genomovars of the *B. cepacia* complex

Genomovar or species	Strain	% Identity[a]	
		CepR	CepI
Genomovar I	ATCC 17759	94 (226/239)[a]	95 (166/173)
B. multivorans	LMG13010	97 (232/239)	ND[b]
B. stabilis	LMG14291	95 (228/239)	90 (156/173)
B. vietnamiensis	PC259	99 (237/239)	96 (167/173)
Genomovar VI	LO6	92 (151/163)	ND

[a] Values in parentheses represent the number of amino acids identical out of the total number of amino acids compared to CepI or CepR from K56-2.
[b] ND, Not determined because not detectable by PCR.

A

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K56-2 CEPI MQTFVHEE.GRLPHELAADLGRYRRRVFVEQLGWALPSANESFERDQFDR    49
DB01 BVII  MLTLLSGRSADLNRETMYQLAKYRHKVFIQELGWTLPTDNG.IEFDNFDH    49

K56-2 CEPI DDTVYVFARNADGDMCGCARLLPTTRPYLLKSLFADLVAEDMPLPQSAAV    99
DB01 BVII  ADTLYVIARDRNGEIVGCGRLLPTDEPYLLGDVFPTLMG.DAALPHAPDV    98

K56-2 CEPI WELSRFAATDDEGG...PGNAEWAVRPMLAAVVECAAQLGARQLIGVTFA    146
DB01 BVII  WELSRFAMSMPRGESLTAEESWQNTRAMMSEIVRVAHAHGANRLIAFSVL    148

K56-2 CEPI SMERLFRRIGIHAHRAGPPKQVDGRLVVACWIDIDPQTFAALGIEPGQAA    196
DB01 BVII  GNERLLKRMGVNVHRAAPPQMIEGKPTLPFWIEIDEQTRAALNLDGLERV    198

K56-2 CEPI RQAIAA................                                202
DB01 BVII  GGVPPKTLRRPDASRALEQSV                                219
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B

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K56-2 CEPR M.ELRWQDAYQQFSAAEDEQQLFQRIAAYSKRLGFEYCCYGIRVPLPVSK    49
DB01 BVIR  MQAWREKY.LNGFATAKSEADVFLEFSADVRALGFDHCSFGLRIPLPISK    49

K56-2 CEPR PAVAIFDTYPDGWMAHYQAQNYIEIDSTVRDGALNTNMIVWPDVDRIDPC    99
DB01 BVIR  PQFMLQSNYPQTWVERYVSQNYFAVDPTVRHGLSRMSPLIWRADSQTQCV    99

K56-2 CEPR PLWQDARDFGLSVGVAQSSWAARGAFGLLSIARHADRLTPAEINMLTLQT    149
DB01 BVIR  QFWEEAGQHGLRHGWCMPSVSRTGAIGLITMVRSGEPIEERELAEKGYQM    149

K56-2 CEPR NWLANLSHSLMSRFMVPKLSPAAGVTLTARDREVLCWTAEGKTACEIGQI    199
DB01 BVIR  SWLANTANYAMSMHLLQRLVPEYTVELTVRERERALQWSAAGKTYAEIGKI    199

K56-2 CEPR LSISERTVNFHVNNILEKLGATNKVQAVVKAISAGLIEAP              239
DB01 BVIR  MHVDDRTVKFHLVNAMRKLNAANKTEAAVKATMLGLLF..              237
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FIG. 2. Computer-generated alignment of the deduced amino acid sequence of CepI from *B. cepacia* K56-2 (accession no. AF019654) (16) with *B. vietnamiensis* BviI (accession no. AF296284) (A) and CepR from *B. cepacia* K56-2 (accession no. AF019654) (16) with *B. vietnamiensis* BviR (accession no. AF296284) (B). Sequence alignments were performed using DNAMAN Sequence Analysis Software (Lynnon Biosoft). Shaded areas indicate identical amino acids.

conserved (>90% identical at the amino acid level) in *B. cepacia* genomovars I and III, *B. stabilis*, and *B. vietnamiensis*. *cepR* was also highly conserved in strains of genomovar VI and *B. multivorans*, suggesting that these strains likely contain the *cepIR* genes but that the *cepI* genes are too divergent to be amplified by the selected primers. Since genomovar VII strains also produce OHL and HHL, it is likely that they have *cepIR* homologues but that these genes may not be as closely related to the *cepIR* homologues in the other genomovars. It is also possible, however, that genomovar VII contains a different

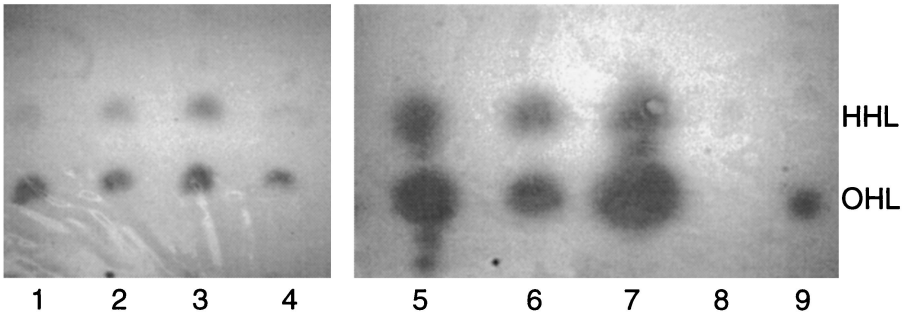

FIG. 3. TLC of acyl-HSLs. Ethyl acetate extracts were chromatographed on C_{18} reversed-phase thin-layer plates developed with methanol-water (60:40, vol/vol). The spots were visualized using the *A. tumefaciens* reporter strain. Samples from each genomovar were chromatographed as follows: lane 1, *B. cepacia* genomovar I (strain CEP509); lane 2, *B. multivorans* (strain C5393); lane 3, *B. cepacia* genomovar III (strain K56-2); lane 4, *B. stabilis* (strain LMG14294); lane 5, *B. vietnamiensis* (strain G4); lane 6, genomovar VI (strain LMG18943); lane 7, genomovar VII (strain CEP996); lane 8, synthetic *N*-hexanoyl-HSL; lane 9, synthetic *N*-octanoyl-HSL.

AHL synthase gene that also directs the synthesis of OHL and HHL in addition to the unidentified molecule with activity in the *A. tumefaciens* reporter assay.

The *bviIR* genes were less related to K56-2 *cepIR* than any of the other *cepIR* homologues identified. Interestingly, only *B. vietnamiensis* contained sequences amplified by the primers designed to *bviIR*. Since *B. vietnamiensis* produces at least two AHL molecules in addition to OHL and HHL, it is likely that *bviIR* are involved in the production of these signals. Further studies are needed to determine the role of the *cepIR* and *bviIR* genes in virulence in the various species of the *B. cepacia* complex.

Nucleotide sequence accession numbers. The nucleotide sequences of the *cepIR* and *bviIR* genes have been deposited in the GenBank and assigned the following accession numbers: AF296284, AF333002, AF333003, AF333004, AF333005, AF333006, AF333007, AF333008, and AF337814.

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