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NOTES

Identification of Quorum-Sensing-Regulated Genes of *Burkholderia cepacia*

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Quorum sensing is a regulatory mechanism (operating in response to cell density) which in gram-negative bacteria usually involves the production of *N*-acyl homoserine lactones (HSL). Quorum sensing in *Burkholderia cepacia* has been associated with the regulation of expression of extracellular proteins and siderophores and also with the regulation of swarming and biofilm formation. In the present study, several quorum-sensing-controlled gene promoters of *B. cepacia* ATCC 25416 were identified and characterized. A total of 28 putative gene promoters show CepR–C_8-HSL-dependent expression, suggesting that quorum sensing in *B. cepacia* is a global regulatory system.

Burkholderia cepacia is a nutritionally versatile, widespread gram-negative bacterium that can be isolated from a variety of sources, including soil, water, and vegetation (5). Originally described as a phytopathogen causing soft rot of onions (4), it has become of wider interest in the field of agriculture due to a remarkable potential as an agent for both biodegradation and biocontrol; thus, it is also being considered a plant growth-promoting rhizobacterium (20, 37). *B. cepacia* has also emerged as an important human opportunistic pathogen for immunocompromised patients and for patients with fibrocystic lung disease (6, 19, 23). Strains of *B. cepacia* have recently been classified in several genomovars which together constitute the *B. cepacia* complex (8, 31). The term "genomovar" refers to a group of strains phenotypically similar but genotypically different. Genomovars II, IV, V, VII, VII, and IX have been named *B. multivorans*, *B. stabilis*, *B. vietnamiensis*, *B. ambifaria*, *B. anthina*, and *B. pyrrocinia*, while genomovar III has been recently named *B. cenocepacia* (53) and genomovar I has not been assigned any further nomenclature and has been referred to as *B. cepacia* (40, 52).

Quorum sensing is a mechanism of communication that bacteria have evolved to correlate gene expression to cell density. This type of communication is mediated by diffusible molecules, originally called autoinducers, which in gram-negative bacteria usually correspond to *N*-acyl-homoserine lactones that differ with respect to the length, saturation, and substitution characteristics of the side chain (for reviews, see references 16, 36, and 55). The quorum-sensing system in the members of the *B. cepacia* complex is very well conserved; it consists of the *cepI*/*R* genes coding for the LuxR family transcriptional activator CepR and a CepI autoinducer synthase (a member of the LuxI family) which synthesizes *N*-octanoylhomoserine lactone (C_8-HSL) and *N*-hexanoylhomoserine lactone (C_6-HSL) (18, 24). The CepR activator binds the C_8-HSL cognate autoinducer at the threshold level, inducing transcriptional activation of target genes (1, 24). Thus far, it has been shown that the CepI/R quorum-sensing system is involved in the negative regulation of the siderophore ornibactin (25) and that it positively regulates extracellular protease and polygalacturonase (PehA) activities, swarming motility, biofilm formation, and *cepI* expression (1, 9, 10, 21, 24)

In the present study, we used an experimental strategy for the identification of quorum-sensing-controlled (QSC) loci in *B. cepacia* ATCC 25146, which belongs to genomovar I. This led to the identification of several CepR–C_8-HSL-regulated gene promoters of this strain. The technique described here is believed to be applicable for other members of the *B. cepacia* complex.

Bacterial strains used in this study include *B. cepacia* ATCC 25416, *B. cepacia* ATCC 25416-I (1), and *Escherichia coli* DH5α (45). Unless otherwise specified, cultures were grown in Luria-Bertani (LB) broth or on LB agar (45). Recombinant DNA techniques involved standard methods (45); restriction and modification enzymes were purchased from New England Biolabs, Inc., Beverly, Mass. Genomic DNA was isolated from cultures grown overnight in LB broth as previously described (3). Analysis of β-galactosidase activity was done as previously described (35, 49); all measurements were done in triplicate, and the mean value is given. An RNeasy kit (Qiagen, Hilden, Germany) was used according to the manufacturer's instructions for purification of RNA from a bacterial culture pellet obtained from stationary-phase cultures. Primer extensions were performed as previously described (45) with the following oligonucleotides: 67PE1 (5′-atgccttgcggcagcgtgatcggggtgc-3′) (complementary to nucleotides 230 to 257 of the P67 sequence), 53PE2 (5′-aaggcccgtcgccgttcgatgcccg-3′) (complementary to nucleotides 259 to 283 of the P53 sequence), and 110PE1 (5′-ccgccagcgacatgaccgcgacgaa-3′) (complementary to nucleotides 191 to 215 of the P110 sequence). The DNA se-

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quencing ladders presented here were generated (using the same oligonucleotides used in the primer extension reaction) by the dideoxy chain termination method (46) with [^{35}S]dATP. All oligonucleotides were purchased from Sigma-Aldrich, St. Louis, Mo.

Identification of CepR–C_8-HSL-regulated promoters of *B. cepacia* ATCC 25416. The experimental strategy used to identify CepR–C_8-HSL-regulated genes was to insert *B. cepacia* genomic DNA fragments digested with different restriction enzymes (*Nru*I, *Hae*III, *Hin*cII, *Alu*I, and *Sau*3AI) within the multiple cloning site of vector pSCR2 (Fig. 1A), scoring for active transcriptional fusions between the genomic fragments and the promoterless reporter gene *lacZ*. Vector pSCR2 also contains the *luxR* gene homologue *cepR* of *B. cepacia* ATCC 25146 that Aguilar et al. have recently shown to have activated gene transcription of a QSC promoter in a heterologous *E. coli* background when C_8-HSL (Fluka; Sigma-Aldrich) was exogenously provided (1). Consequently, clones were scored for β-galactosidase activity dependent upon the presence of C_8-HSL.

Plasmid vector pSCR2 was constructed as follows: pBIR (1) (as the template) and two oligonucleotides (CEPRD2 [5′-ctcc atgggtaacggtttcttgatcaac-3′] and CEPR-RA [5′-tggcatgccctcgtt cgaggtcagggcg-3′]) were used to amplify the *cepR* gene as a 1,347-bp DNA fragment. Consequently, the PCR-amplified product was digested and cloned as an *Nco*I-*Sph*I fragment into the corresponding sites of pQF50 (12), thus yielding pSCR2. Several restriction enzyme sites were maintained upstream from the promoterless *lacZ* gene (Fig. 1).

To test the ability of pSCR2 to detect CepR–C_8-HSL QSC gene promoters in *E. coli*, a 250-bp *Bam*HI-*Hin*dIII fragment (obtained by PCR with pBIR as the template and the oligonucleotides CEPID [5′-ggtcgcgctcgaagctttcgttcgcc-3′] and CEPIR [5′-ccccgcggatccacgtcctgatcggcgtca-3′]) containing the promoter region of *cepI*, a known *B. cepacia* QSC gene (25), was cloned upstream from the promoterless *lacZ* gene, yielding pSCR2C. In this control experiment, we observed 15-fold-increased transcriptional activation in *E. coli* (pSCR2C) (measured as β-galactosidase activity) when C_8-HSL was externally provided (Table 1). On other hand, when the same PCR product, containing the *cepI* promoter, was cloned in the corresponding sites of vector pQF50, yielding pSCon, no β-galactosidase activity was detected regardless of the presence or absence of C_8-HSL, thus confirming the requirement of CepR for the activation of transcription of the *lacZ* gene in pSCR2 (Fig. 1B and Table 1).

DNA ligations between pSCR2 and the genomic restriction DNA fragments from *B. cepacia* (obtained using the restriction enzymes *Nru*I, *Hae*III, *Hin*cII, *Alu*I, and *Sau*3AI independently) were transformed in *E. coli* DH5α and plated on selective medium containing 100 μg of ampicillin (Sigma-Aldrich)/ml, 20 μg of X-Gal (Sigma-Aldrich)/ml, and 100 nM C_8-HSL. From the various cloning experiments we obtained approximately 50,000 colonies, and of these, approximately 2,400 had turned blue in the selective plates, indicating that a promoter most probably had been cloned upstream from the promoterless *lacZ* gene in pSCR2. To identify cloned gene promoters that had been activated in a CepR–C_8-HSL-dependent way, all 2,400 clones were used in cross-streaking experiments on plates containing 100 μg of ampicillin/ml and 20 μg of X-Gal/ml in close proximity to the parent *B. cepacia* ATCC

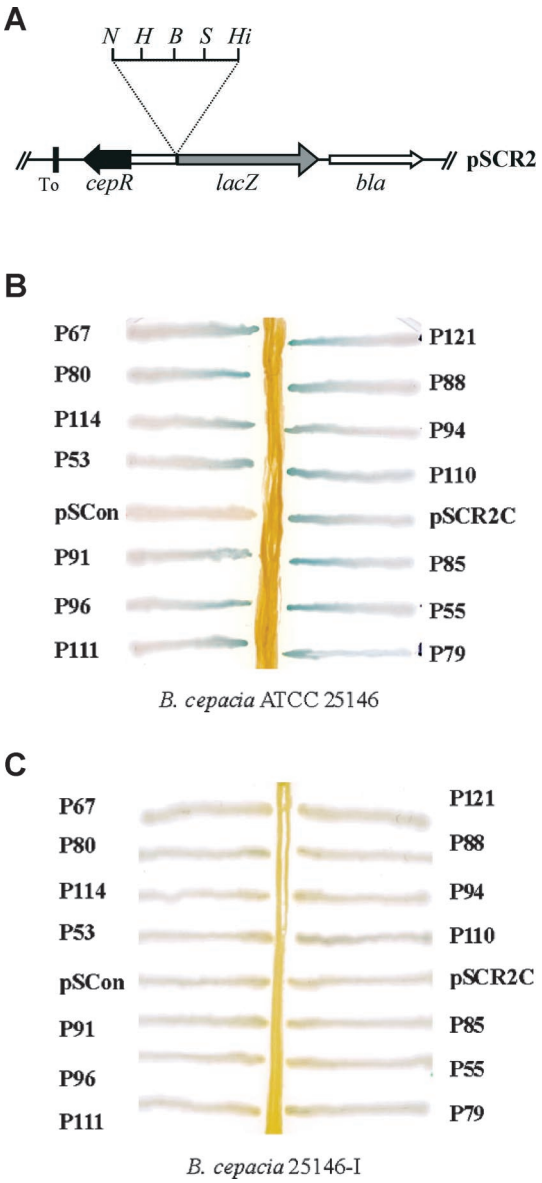

FIG. 1. (A) Schematic diagram (not to scale) of the *cepR*-based cloning plasmid used to identify QSC genes. Plasmid pSCR2 contains the *B. cepacia* ATCC 25146 *cepR* gene cloned in the broad-host-range plasmid pQF50 (12). The genetic components are as follows: *cepR*, the gene encoding the transcriptional activator CepR; *lacZ*, a promoterless β-galactosidase gene; T_0, a transcriptional *trpA* terminator; *bla*, an ampicillin resistance marker. The indicated restriction sites in the polylinker (N, *Nco*I; H, *Hin*cII; B, *Bam*HI; S, *Sma*I; Hi, *Hin*dIII) were used to clone DNA fragments obtained from genomic DNA of *B. cepacia* ATCC 25416 digested with compatible-end restriction enzymes. Positive clones on selective plates in the presence of C_8-HSL were identified as blue colonies (see text for details). (B) *E. coli* clones were cross-streaked in close proximity to *B. cepacia* ATCC 25416 in LB plates containing 100 μg of ampicillin/ml and X-Gal and were incubated overnight at 30°C. The blue coloration developed only in proximity to the *B. cepacia* strain. pSCR2C and pSCon were the positive and negative controls, respectively. (C) *E. coli* clones were cross-streaked in close proximity to *B. cepacia* 25416-I on plates containing 100 μg of ampicillin/ml and X-Gal and were incubated overnight at 30°C. No blue coloration was observed either with clones or with the pSCR2C positive control, thus confirming the strict C_8-HSL-dependent expression of the reporter β-galactosidase gene.

TABLE 1. Putative ORFs regulated by quorum sensing in *Burkholderia cepacia*

Clone	Enzyme[a]	Quorum sensing-regulated ORF[b]	Identity[c] (%)	Activity with[d]:		
				C_8-HSL$^+$ CepR$^+$	C_8-HSL$^-$ CepR$^+$	C_8-HSL$^+$ CepR$^-$
67	N	AceB, malate synthase A from *R. solanacearum*	79	28	1	1
80	N	DpsA[e], stress-induced DNA binding protein from *B. pseudomallei*	92	27	1	1
114	N	Hypothetical protein from *B. fungorum*	72	26	1	2
53	N	AidA from *R. solanacearum*	56	17	2	1
91	A-Hi	PpiaseB, peptidil-prolyl *cis*/*trans* isomerase from *R. solanacearum*	80	24	1	1
96	A-N	CeoA from *B. cepacia* K61-3	32	24	1	2
111	Hi-N	Short chain dehydrogenase-reductase from *P. putida* KT2440	68	25	2	2
121	Hi-N	Putative NrdA ribonucleoside reductase 1 from *R. solanacearum*	82	15	1	1
88	A-N	Unknown		15	1	1
94	A-N	Unknown		19	1	3
110	Hi-N	Putative ABC amino acid transporter from *R. solanacearum*	70	15	1	1
85	A-N	Unknown		17	1	1
55	N	Putative ABC sugar transporter from *R. solanacearum*	89	11	1	1
79	Ha	AT2 DNA binding protein from *Oryza sativa*	40	16	1	2
69	N	ExbB from *Bordetella bronchiseptica*	38	9	1	1
103	A-Hi	CatA2, catechol 1,2-dioxygenase from *Burkholderia* sp. strain TH2	73	13	1	1
81	A	PBP-3 from *R. solanacearum*[f]	66	15	1	1
56	N	AcoD, acetaldehyde dehydrogenase II from *Alcaligenes eutrophus*	73	7	1	1
57	S	Probable porin transmembrane protein from *R. solanacearum*	41	10	1	2
105	A-N	MviN[f] from *Salmonella tiphymurium* LT2	52	12	1	1
59	S	Unknown		6	1	1
68	N	TrkA potassium uptake protein from *B. subtilis*	39	10	1	1
130	Hi-N	Unknown		9	1	1
122	Hi-N	Unknown		8	2	2
135	A-Hi	Human RNA binding protein BAA83713	36	9	1	1
15	Hi	PilA, pilus subunit from *Caulobacter crescentus* CB15	53	6	1	1
38	A	YwnB from *B. subtilis*	36	5	1	1
1	A	Unknown		6	1	1
pSCR2C	NA[g]	*cepI* promoter 250 bp *Bam*HI-*Hin*dIII cloned in pSCR2	NA	15	1	1
pSCon	NA	*cepI* promoter 250-bp *Bam*HI-*Hin*dIII cloned in pQF50	NA	1	1	1

[a] Restriction enzymes used to digest *B. cepacia* ATCC 25416 genomic DNA: N, *Nru*I; Ha, *Hae*III; Hi, *Hin*cII; A, *Alu*I; S, *Sau*3AI.
[b] For some of the ORFs, it was not possible to find identity to any ORF from the databases and those are shown as unknown.
[c] Amino acid identity to the complete ORF obtained from the *B. cepacia* J2315 sequence database.
[d] Activity expressed in Miller units determined from overnight cultures in the presence (+) and/or absence (−) of C_8-HSL and CepR.
[e] Homology found downstream the P80 sequence, derived from the *B. cepacia* J2315 sequence.
[f] The identified sequence is inside the ORF (see text and Figure 2).
[g] NA, not applicable.

25416 strain as an exogenous source of C_8-HSL. Of these 2,400 clones, 28 clones had turned blue only in close proximity to *B. cepacia* ATCC 25416, as shown in part in Fig. 1B. To confirm the C_8-HSL dependence of expression, the clones were also cross-streaked with the quorum-sensing mutant *B. cepacia* 25416-I, which does not produce HSL molecules (1), and as can be seen in Fig. 1C, no blue coloration was visible. Consequently, it was postulated that these 28 clones contained putative promoters of *B. cepacia* ATCC 25416 driving the transcription of the reporter *lacZ* gene in a CepR–C_8-HSL-dependent manner in the *E. coli* background. This was further confirmed for each clone by performing β-galactosidase assays (35, 49) in the presence or absence of C_8-HSL, and as shown in Table 1, there was a 5- to 25-fold activation of transcription when C_8-HSL was present in the medium. To confirm that transcriptional activities driven from the cloned DNA fragments were, apart from being C_8-HSL dependent, also CepR dependent, each DNA fragment containing the putative QSC promoters was cloned in the original pQF50 vector as a *Sal*I/*Hin*dIII or *Bam*HI/*Hin*dIII fragment. As shown in Table 1, the clones devoid of the CepR gene did not display activation of transcription in the presence of C_8-HSL. It was concluded that activation of transcription from the cloned DNA fragments in *E. coli* was dependent on the presence of C_8-HSL and the CepR protein, thus strongly indicating that they contain QSC promoters of *B. cepacia*.

Characterization of clones that activated transcription in a CepR–C_8-HSL-dependent way. The sequence of the *B. cepacia* ATCC 25416 genomic DNA present in all the 28 clones was determined, and it was subsequently compared (using the BLASTN program) to sequences in the sequenced genome of *B. cepacia* J2315 (http://www.sanger.ac.uk/Projects/B_cepacia/). We observed that the majority (20 of 28) of the clones displayed very high (more than 80%) identity at the DNA level with a region of the genome. Moreover, we also observed that these clones included a part of an open reading frame (ORF) or were just upstream from one. In contrast, seven clones had rather small inserts (ranging from 57 to 294 bp) and it was not possible to detect any significant homologies with regions of the *B. cepacia* J2315 genome or any other DNA sequence available in data banks. It is therefore possible that these clones contained intergenic regions of DNA or represented promoters of QSC genes which were strain specific, and further characterization is necessary to determine which gene(s) these

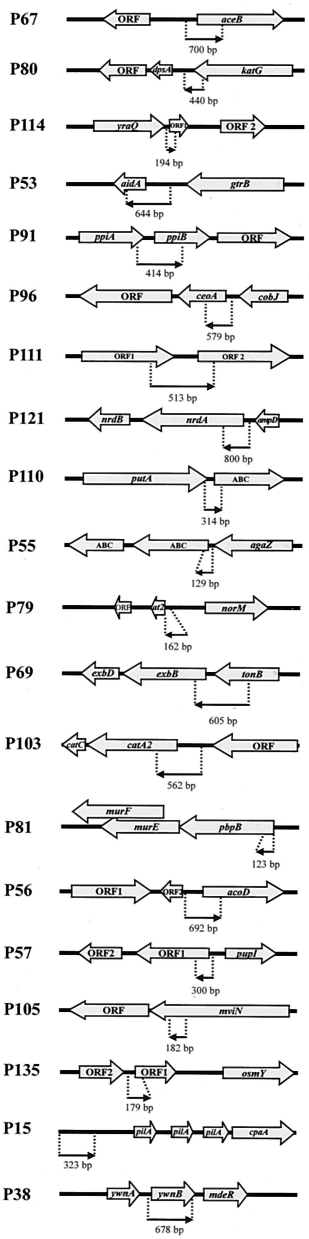

FIG. 2. Characterization of identified clones containing putative CepR–C_8-HSL gene promoters. The DNA sequence of each identified clone was compared (using the BLASTN program [http://www.sanger.ac.uk/Projects/B_cepacia]) to sequences in the genome of *B. cepacia* J2315. For those clones that showed a high degree of identity at the DNA level (see text), a region of the genome surrounding 2 kbp of each sequence was subsequently annotated. In the majority of the cases, it was possible to identify a homologue of a gene coding for a protein of known function. In such cases, the names of the respective genes are indicated inside of the putative ORFs (represented as thick gray arrows). A black arrow represents the size, position, and orientation of the identified insert in each clone (in scale with each clone). Within some genetic regions shown, hypothetical proteins of unknown function were found (here designated ORF). Such was the case with P67 (ORF represents what was probably a *lysR* family transcriptional regulator from *R. solanacearum*), P80 (ORF represents a 4-hydroxybenzoate octaprenyl transferase from *Xanthomonas axonopodis*), P114 (ORF1 represents a hypothetical protein from *B. fungorum*; ORF2 represents a probable transcriptional regulator, PA1627, from *P. aeruginosa*), P111 (ORF2 represents a short chain dehydrogenase-reductase from *Pseudomonas putida*, KT2440; ORF1 represents a hypothetical protein, PA1829, from *P. aeruginosa*), P79 (ORF represents a protein of unknown function), P103 (ORF represents a protein of unknown function), P56 (ORF1 represents a probable efflux membrane protein from *Mesorhizobium loti*; ORF2 represents a probable nucleoside triphosphate pyrophosphohydrolase from *Agrobacterium tumefaciens*), P57 (ORF1 represents a probable porin transmembrane protein from *R. solanacearum*; ORF2 represents a putative lipoprotein from *R. solanacearum*), P105 (ORF represents a hypothetical protein from *R. solanacearum*), and P135 (ORF1 represents a human RNA binding protein; ORF2 represents a putative small heat shock protein from *R. solanacearum*). See text for details of all other ORFs mentioned.

putative identified promoters regulate. Two identified clones appeared to represent intragenic regions; thus, they do not seem to have been proximal to promoter sequences. Some promoters in bacteria were found intragenically; however, the possibility that these two clones gave false-positive results cannot be excluded. A BLASTP analysis of the 20 putative ORFs postulated to be under the control of the cloned quorum-sensing-dependent promoters is shown in Table 1. The seven clones without significant homologies are indicated as unknown. In silico analysis of the 20 clones from Table 1 whose sequences displayed significant identity to regions of the *B. cepacia* J2315 genome is presented in Fig. 2. The size of the insert and the corresponding region (and adjacent loci) of high identity in the genome of *B. cepacia* J2315 (with the gene(s) identified [where possible] in that region) are depicted in that figure.

To further verify whether the cloned fragments contained promoters and whether they were QSC in the parent strain, we carried out mapping (using total mRNA from wild-type *B. cepacia* ATCC 25416 and *cepI* knockout mutant derivative *B. cepacia* 25416-I) of the mRNA 5′ end by reverse transcription of clones P67, P53, and P110. A primer was designed near the far end of the clone (see above) and also served to generate the corresponding sequence ladder (Fig. 3). Primer extension analysis (as depicted in Fig. 3) revealed the presence of a transcript initiation point which was in part dependent on the quorum-sensing system, since the transcript abundance decreased significantly in the *cepI* mutant, demonstrating that the QSC loci identified in *E. coli* are also QSC in *B. cepacia*.

As described above and depicted in Fig. 2, several ORFs are postulated to be under CepR-C_8-HSL control. Among the findings pertaining to such ORFs were the following. (i) Sequence analysis of clone P67 revealed the presence of a malate synthase, an enzyme belonging to the glyoxylate cycle (44) which has been associated with virulence in *Mycobacterium tuberculosis* and *Candida albicans* (29, 33). (ii) Sequence analysis of clone P80 did not reveal the presence of any putative ORF; however, a DpsA homologue was found in the genome of *B. cepacia* J2315 around 200 bp downstream from the identified sequence (Fig. 2). DpsA is a ferritin which is induced under stress conditions such as periods of nutrient starvation or oxidative stress (28, 42). (iii) Clone P53 contained the promoter of the *aidA* homologue, which encodes AidA (autoinducer dependent), a protein of unknown function whose expression has been shown to be regulated by quorum sensing in *Ralstonia solanacearum* (15) and shown recently also to be regulated by quorum sensing in *B. cepacia* H111 (43). (iv) Clone P91 contained the promoter of a gene encoding a peptidyl-

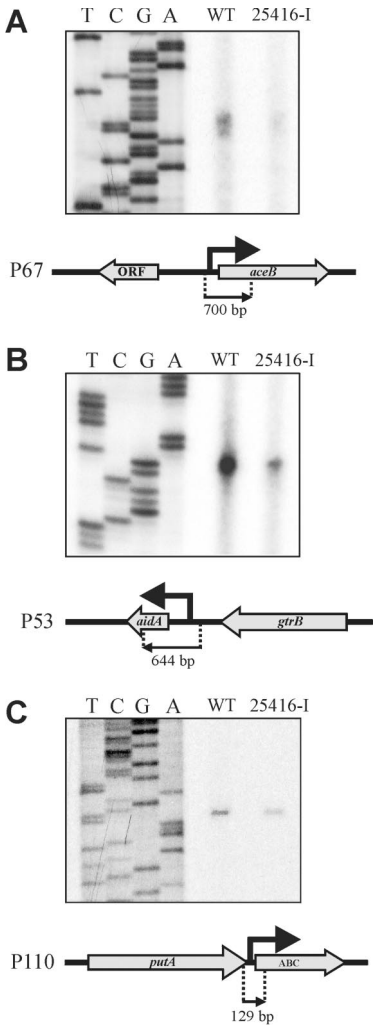

FIG. 3. Primer extension analysis of three identified QSC promoters. Primer extension mapping of the mRNA 5′ end of clones P67 (A), P53 (B), and P110 (C) is shown. Total RNA was isolated from *B. cepacia* ATCC 25416 (WT) and the *cepI* mutant derivative (25416-I). Primer extensions were performed using 15 μg of RNA, and extension products were run adjacent to the sequencing ladder generated with the same primer used for reverse transcription. The approximate position of the mRNA 5′ end is depicted for each clone and indicated by an arrow.

prolyl *cis*/*trans* isomerase (44) which has been described as an essential virulence factor for *Legionella pneumophila* (14, 30) and the expression of which has been associated with the response in *Pseudomonas aeruginosa* under extreme stress conditions (13). (v) Clone P96 was found to contain a promoter controlling an ORF similar to that for CeoA protein AAB58160 (believed to be member of a putative efflux operon associated with multiple antibiotic resistance). (vi) Clone P57 is believed to contain a promoter regulating the expression of a porin gene (44). (vii) Clone P55 appeared to have a promoter upstream from an ORF encoding an ABC transmembrane transporter of sugars, and clone P110 appeared to have a promoter upstream from an ORF encoding a transporter of amino acids (44). (viii) A catechol 1,2-dioxygenase homologue (51) and its promoter were detected in clone P103. (ix) A promoter controlling an ORF homologue of ribonucleoside reductase 1 (44) was observed in clone P121. (x) A promoter for an acetaldehyde dehydrogenase II (41) was present in clone P56 and one for short chain dehydrogenase-reductase (38) was present in clone P111. (xi) In clone P69 a promoter controlling the expression of an ExbB homologue, which is a member of the cytoplasmic membrane complex TonB, ExbB, and ExbD (involved in the transport of iron siderophores, haem-haemin, transferrin, and vitamin B_{12} in various gram-negative bacteria), was identified (39). Interestingly, an ExbB homologue in *P. aeruginosa* has also recently been described as QSC (47). (xii) In clones P79 and P135, the identified sequences were apparently driving the transcription of an ORF sharing some identity with a DNA (34) and with RNA binding protein BAA83713, respectively (Table 1). (xiii) In the case of clone P68, the DNA did not share a high level of identity with the genomic DNA of *B. cepacia* J2315, so the whole fragment (686 bp) was analyzed and a putative promoter was found to be followed by a putative ORF sharing partial (39%) identity with TrkA potassium uptake protein from *Bacillus subtilis* (50) (data not shown). (xiv) DNA downstream from clone P15 was localized to an ORF coding for a PilA homologue (48) required for virulence and twitching motility in *P. aeruginosa* (17) and *R. solanacearum* (27); interestingly, *pilA* in *P. aeruginosa* is not regulated by quorum sensing (2a). (xv) In clone P38, a putative promoter was found controlling a YwnB homologue, a protein of unknown function in *B. subtilis* (11). Interestingly, downstream from this sequence there was a homologue of MdeR, a protein member of the Lrp family of transcriptional regulators (22).

In the case of clone P81, it was established that the identified clone contained highly identical DNA sequences within an ORF coding for a putative penicillin binding transmembrane protein from *R. solanacearum* (44) (Table 1) and, interestingly, MurE and MurF homologues were found downstream from this sequence. A cluster in *P. aeruginosa* with similar organization has been described that is part of a peptidoglycan biosynthesis pathway (2, 26). It is not known whether there is a functional promoter in this clone; future work will determine whether a gene promoter is present within this putative ORF. Similarly, in clone P105 the identified putative promoter was localized on the basis of homology searches inside an ORF with identity to the virulence factor MviN (32) followed by searches inside another ORF encoding a putative protein of unknown function (Fig. 2).

Using the screening performed in the present study, we identified 28 different putative promoters of genes positively regulated by quorum sensing; to our knowledge, this is the first report of a molecular approach aimed at characterizing the quorum-sensing regulon in *B. cepacia*. We do not believe that this number represents all the promoters in the quorum-sensing regulon of *B. cepacia*; in fact, known CepR-C_8-HSL promoters such as *cepI* (1) were not identified in this screening. Further investigations are needed to confirm the total number of possible QSC loci; the results presented here are highly suggestive that quorum sensing is a global regulatory system in *B. cepacia*. This latter observation has also recently been made by Riedel et al. (43), whose use of proteomics determined that about 6% of the proteins in *B. cepacia* H111 are subjected to control by quorum sensing. Similarly, identification of QSC

genes via the use of microarrays has determined that in *P. aeruginosa* about 6% (above 200) of the genes present in the chromosome are affected either positively or negatively by quorum sensing (47, 54). It must be stressed that for the complete identification and characterization of a regulon in bacteria several different approaches must be conducted, since every methodology has limitations, as sensibility or growth conditions can possibly mask the expression of genes that otherwise could be easily detected (7). We tried to search for a putative *cep* box representing a putative *lux* box-like binding sequence in which CepR-C_8-HSL binds and regulates gene expression; however, this proved difficult, as these boxes do not have many nucleotides which are conserved and to date there is no CepR binding region yet established. Interestingly, the methodology used in the present work does not depend on the expression level of the gene of interest in the *B. cepacia* background and it detects CepR-C_8-HSL directly activated gene promoters in the *E. coli* background. The results of the investigations of CepR–C_8-HSL-dependent expression in *E. coli* and the cross-streaking experiments with *B. cepacia* offer convincing evidence that in the identified fragments there is a quorum-sensing-modulated promoter. One limitation is that only positive regulation by quorum sensing is easily detectable using this screening system. Future work will also need to focus on the possible negative regulation of gene expression by quorum sensing in *B. cepacia*. Finally, since CepR homologues among the *B. cepacia* complex share 93 to 97% amino acid identity (data not shown), it is very likely that the same pSCR2 vector can probably be used in a way similar to that described in this study to obtain information about QSC genes (CepR–C_8-HSL dependent) for strains of the different genomovars in the *B. cepacia* complex and possibly also for other bacterial species which produce HSL molecules that can be recognized by CepR.

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