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Naturally Occurring Class A ß-Lactamases from the *Burkholderia cepacia* Complex[▽]

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Chromosomally encoded ß-lactamases from the *Burkholderia cepacia* complex species (formerly *Pseudomonas cepacia*) were characterized. Cloning and sequencing identified an Ambler class A ß-lactamase (PenB) from *B. cenocepacia*. It shares 82% amino acid identity with the PenA ß-lactamases previously identified from *B. multivorans* 249. Its expression was dependent upon a LysR-type regulatory protein. Its narrow-spectrum hydrolysis activity mostly included penicillins but also included expanded-spectrum cephalosporins and aztreonam at lower levels. In that study, Pen-like ß-lactamases (PenC, PenD, PenE, PenF) that shared 63 to 92% identity with PenB from *B. cenocepacia* were identified from other *Burkholderia* species. The corresponding ß-lactamase genes might be used as genetic tools for accurate *Burkholderia* species identification.

Over the last two decades, *Burkholderia cepacia* (previously *Pseudomonas cepacia*) has been recognized as an ubiquitous and opportunistic pathogen of increasing importance, particularly in nosocomial infections in immunocompromised hosts and cystic fibrosis (CF) patients (20, 30, 32, 35). The *Burkholderia cepacia* complex is divided into at least 10 different closely related species: *B. cepacia*, *B. multivorans*, *B. cenocepacia*, *B. stabilis*, *B. vietnamiensis*, *B. dolosa*, *B. ambifaria*, *B. anthina*, *B. pyrrocinia*, and *B. ubonensis* (genomovars I, II, III, IV, V, VI, VII, VIII, IX, and X, respectively) (21, 31, 40). Some epidemic clones have been described as sources of human infections in Canada, the United Kingdom, and France (12, 14, 15, 16, 33); and *B. cepacia* complex strains are also commonly found in the environment in soil (10, 18), water (39), and onions (6). The prevalence of isolation of *Bulkholderia* sp. strains in CF patients in France is about 3.1%, with *B. cenocepacia* (genomovar III) and *B. multivorans* (genomovar II) being the most frequently isolated bacterial species (3, 21).

The treatment of *B. cepacia* infections is difficult, since *B. cepacia* species often have high-level intrinsic resistance to many antibiotics, including ticarcillin, most cephalosporins, aminoglycosides, fosfomycin, and the polymyxins (5, 41). However, Nzula et al. have noticed a heterogeneity of intrinsic antibiotic resistance patterns among the members of the *B. cepacia* complex that is likely related to the genomovar type (25).

Resistance to β-lactam antibiotics in isolates of the *B. cepacia* complex has been related to a chromosomal and inducible β-lactamase which has been falsely identified as an AmpC enzyme (28). Then, Trépanier et al. (36) described a chromosomal Ambler class A β-lactamase (PenA) from *B. cepacia* 249. The PenA ß-lactamase possesses a narrow-spectrum profile, and it is regulated by a LysR-type transcriptional regulator, PenR. This negative regulator is responsible for the inducibility of PenA expression (36).

The aim of the study described here was to identify the putative ß-lactamases produced by strains belonging to the *B. cepacia* complex and, in particular, to characterize the ß-lactamase determinants from *B. cenocepacia*, which is the main *Burkholderia* species identified from CF patients in France. The newly identified ß-lactamase, PenB, was studied for (i) its hydrolysis activity toward ß-lactams, (ii) the inducibility of its expression, and (iii) the distribution of its gene among *B. cepacia* complex isolates.

MATERIALS AND METHODS

Bacterial strains and plasmids. Eight *B. cenocepacia* clinical isolates obtained from CF patients and belonging to genomovar III were obtained from C. Segonds, Toulouse, France. They had been recovered from distinct hospitals in distinct cities in France. Two *Burkholderia multivorans* (genomovar II), one *Burkholderia stabilis* (genomovar IV), one *Burkholderia pyrrocinia* (genomovar IX), and two *Burkholderia vietnamiensis* (genomovar V) isolates identified by the use of molecular tools (38), recovered from French CF patients, and provided by the collection Observatoire Cepacia (Laboratoire de Bacteriologie-Virologie-Hygiene, Hôpital Rangueil, Toulouse, France) were included in the study. *Escherichia coli* TOP10 (Invitrogen, Life Technologies, Cergy-Pontoise, France) was used as the host for the cloning and expression experiments. Kanamycin-resistant plasmid pBK-CMV was used as the cloning vector. Bacterial cultures were grown in Trypticase soy (TS) broth at 30°C and 37°C for 18 h for the *Burkholderia* spp. and *E. coli*, respectively.

Antimicrobial agents and MIC determinations. The antimicrobial agents used in this study were obtained from pure laboratory powders and were used immediately after their solubilization. The agents and their sources were as follows: amoxicillin (amoxicilline), clavulanic acid, ceftazidime, and ticarcillin, GSK (Nanterre, France); aztreonam and cefepime, Bristol-Myers Squibb (Paris-La-Défense, France); cephalothin (cefalotin) and moxalactam (latamoxef), Eli Lilly (Saint-Cloud, France); piperacillin and tazobactam, Lederle (Oullins, France); sulbactam, Pfizer (Orsay, France); and cefotaxime and cefpirome, Hoechst-Roussel (Paris, France). MICs were determined by the microdilution and Etest techniques, as described previously (26). The results of susceptibility testing were interpreted according to the guidelines of the CLSI (8).

Cloning experiments and PCR experiments. For each PCR experiment, 500 ng of total DNA was used in a standard PCR mixture. By using the total DNA of the different *Burkholderia* sp. isolates, PCR amplifications of the bla_{Pen}-like genes were performed with either external primers Pre-PenA-1 and Pre-PenA-2 or

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TABLE 1. Primers used in this study

Primer	Gene, primer type	Sequence (5′ to 3′)
Pre-PenA-1	bla_{PEN-A}, external	GCGTTCCGCATTCGTTTCGC
Pre-PenA-2	bla_{PEN-A}, external	TGGCCGCTCACGCGAG CGTC
PenA-1	bla_{PEN-A}, internal	TTACACCATTCTCGGC ACGG
PenA-2	bla_{PEN-A}, internal	AGAAACAAGGAACTGT TGGC
Pre-PenB-1	bla_{PEN-B}, external	CTCATCGAAACGTCGA ACCC
Pre-PenB-2	bla_{PEN-B}, external	CGTCTGCGTGTAGTAC ACGG
PenB-1	bla_{PEN-B}, internal	GTCTCGATAGTGCTGT CTCG
PenB-2	bla_{PEN-B}, internal	TTACGTGCCAGTTGCT GACG

internal primers PenA-1 and PenA-2 (Table 1), designed from the published *penA* sequence. In a second step, other PCR amplifications were performed with either external primers Pre-PenB-1 and Pre-PenB-2 or internal primers PenB-1 and PenB-2 (Table 1), which were newly designed from the identified *penB* sequence.

Total DNAs of the *B. cepacia* strains were partially digested with the Sau3AI restriction enzyme, ligated into the BamHI site of linearized plasmid pBK-CMV, and transformed into reference strain *E. coli* TOP10, as described previously (13). Recombinant plasmids were selected on TS agar plates containing amoxicillin (50 μg/ml) and kanamycin (30 μg/ml).

DNA sequencing and protein analysis. The PCR-generated fragments, purified by using QIAquick PCR purification spin columns (Qiagen, Courtaboeuf, France), and both strands of the inserts from the recombinant plasmids were sequenced on an ABI 3100 automated sequencer (Applied Biosystems, Les Ulis, France). The nucleotide and deduced protein sequences were analyzed with software available over the Internet at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov). Dendrograms were derived from a multiple-sequence alignment by a parsimony method with the phylogeny package PAUP (Phylogenetic Analysis Using Parsimony), version 3.0 (34).

IEF analysis and induction studies. Isoelectric focusing (IEF) analysis was performed with a pH 3.5 to 9.5 Ampholine polyacrylamide gel (GE Healthcare, Orsay, France) with culture extracts of the different *B. cepacia* complex isolates and of recombinant *E. coli* TOP10 harboring plasmid pBcSau13. The inducibility of the ß-lactamase was tested in TS broth at 37°C with imipenem (0.6 μg/ml) as the ß-lactam inducer, and hydrolysis was measured with 100 μM benzylpenicillin as the substrate. The ß-lactamase activity was defined as the hydrolysis of 1 μmol of benzylpenicillin per min by 1 U of enzyme. The total protein content was measured with bovine serum albumin as the standard (DC protein assay kit; Bio-Rad).

TABLE 3. Amino acid substitutions in PenB-like proteins

Protein	Substitution at the following allele/position[a]:						
	80	119	138	149	173	182	214
PenB1	V	K	A	T	A	A	R
PenB2	I	T	S	A	V	T	L
PenB3		T			V		
PenB4		T					

[a] Note that the position does not refer to the Ambler nomenclature but refers to the positions in the PenB-like sequences. GenBank accession numbers are as follow; EU872211 for PenB1, FJ386399 for PenB2, FJ386401 for PenB3, and FJ386402 for PenB4.

ß-Lactamase purification and IEF analysis. Cultures of recombinant *E. coli* TOP10(pBcSau13) were grown overnight at 37°C in 4 liters of Trypticase soy broth containing amoxicillin (100 μg/ml) and kanamycin (30 μg/ml). ß-Lactamase was purified by ion-exchange chromatography, as described previously (26). Briefly, the ß-lactamase extract was sonicated, cleared by ultracentrifugation, treated with DNase, and dialyzed against 20 mM bis-Tris buffer (pH 8). This extract was loaded onto a Q-Sepharose column, and the ß-lactamase-containing fractions were eluted with a linear NaCl gradient from 0 to 0.5 mM. The fractions containing the highest ß-lactamase activity were dialyzed against 20 mM bis-Tris buffer (pH 5.5) and were subsequently reloaded onto the preequilibrated Q-Sepharose column. The ß-lactamase activity was recovered in the flowthrough, and then the extract was concentrated with an ultrafiltration filter tip (Sartorius, Göttingen, Germany). The purity of the enzyme was estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis (11).

Kinetic studies. Purified ß-lactamase was used for determination of kinetic parameters (k_{cat}, K_m), which was performed at 30°C in a reaction buffer made of 100 mM phosphate (pH 7.0) (18, 24). The initial rates of hydrolysis of the ß-lactams were determined with a UV spectrophotometer, as described previously (13). The 50% inhibitory concentration (IC_{50}) was determined as the clavulanate or tazobactam concentration that reduced the rate of hydrolysis of 100 μM piperacillin by 50% under conditions in which the enzyme was preincubated with various concentrations of inhibitor for 3 min at 30°C before addition of the substrate (13).

Nucleotide sequence accession numbers. The nucleotide sequences reported in this paper have been submitted to the EMBL/GenBank nucleotide sequence databases under the following accession numbers: EU872211 for PenB1, FJ386399 for PenB2, FJ386401 for PenB3, FJ386402 for PenB4, FJ457906 for PenC, FJ457907 for PenD, and FJ386400 for PenE.

TABLE 2. MICs of β-lactams for *B. cepacia* complex isolates 07-34, 09-54, 212, and 5007902; *E. coli* TOP10 harboring recombinant plasmid pBcSau13 expressing the PenB1 β-lactamase; and the *E. coli* TOP10 reference strain

β-Lactam(s)[a]	MIC (μg/ml)					
	B. cenocepacia 07–34 (PenB1)	*B. cenocepacia* 09–54 (PenB2)	*B. cenocepacia* 212 (PenB3)	*B. cenocepacia* 5007902 (PenB4)	*E. coli* TOP10(pBcSau13) (PenB1)	*E. coli* TOP10
Amoxicillin	512	512	512	512	512	4
Amoxicillin+ CLA	512	512	512	512	16	4
Ticarcillin	512	512	512	512	512	4
Piperacillin	4	512	128	4	8	2
Piperacillin + TZB	4	256	128	4	4	2
Cefuroxime	16	512	512	32	16	8
Ceftazidime	4	32	16	0.25	1	0.5
Cefotaxime	1	512	512	4	0.25	0.06
Cefepime	32	64	64	1	0.125	0.06
Cefoxitin	512	512	512	512	8	8
Aztreonam	4	512	512	16	0.5	0.06
Imipenem	8	16	16	8	0.5	0.25

[a] CLA, clavulanic acid at a fixed concentration of 4 mg/ml; TZB, tazobactam at a fixed concentration of 4 mg/ml.

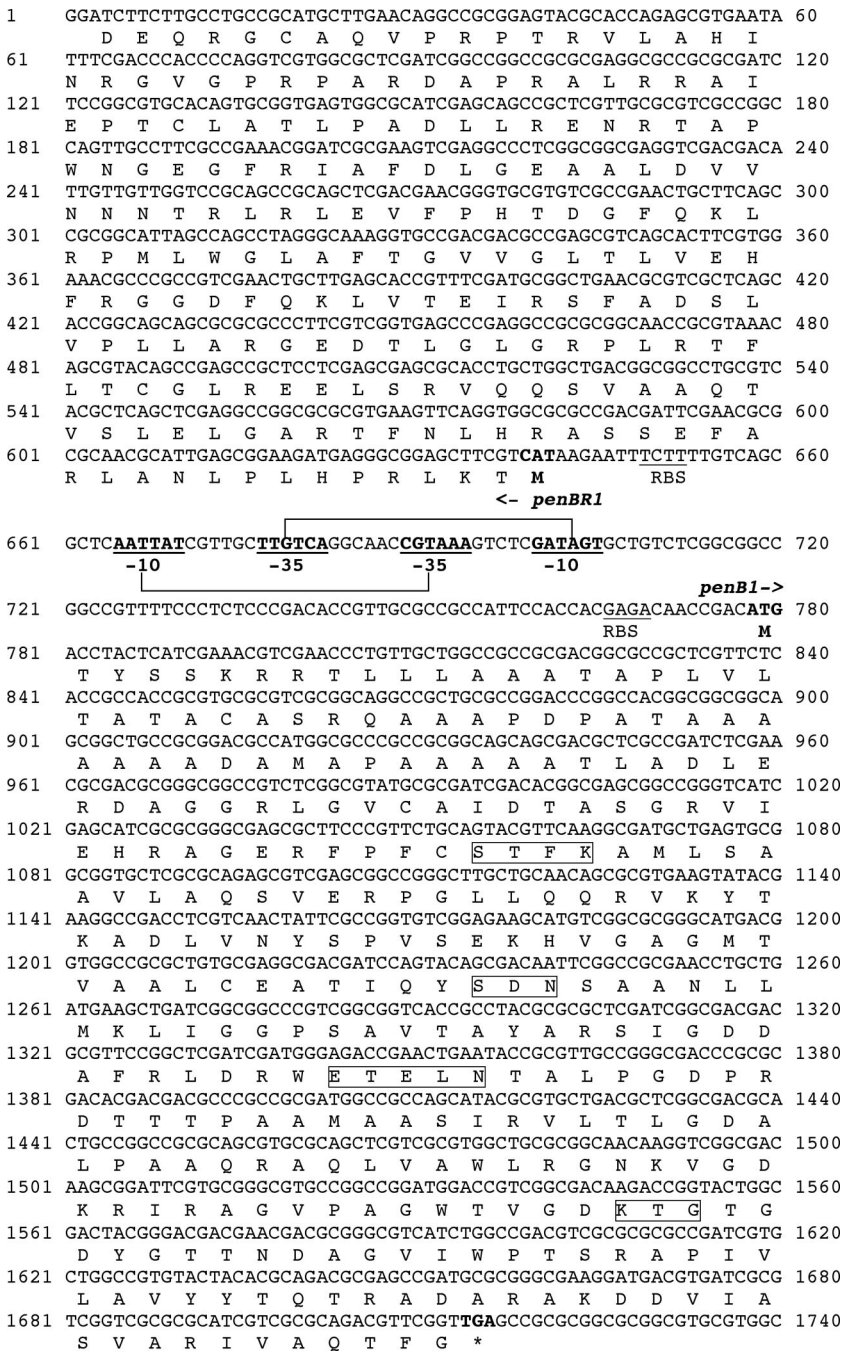

FIG. 1. Nucleotide sequence of the 1,740-bp fragment of pBcSau13 containing the *penB1*- and *penRB*-coding regions. The deduced amino acid sequences are designated in single-letter code. The putative and overlapped promoter sequences are represented by −35 and −10 regions (in boldface and underlined). The start and stop codons of these genes are also in boldface, and the putative RBS sequence is underlined. The critical motifs for class A ß-lactamases are bracketed.

RESULTS AND DISCUSSION

Susceptibility testing. The eight *B. cenocepacia* isolates showed various profiles of resistance to ß-lactams. However, they were all highly resistant to aminoglycosides, tetracycline, fosfomycin, and trimethoprim. *B. cenocepacia* strains 07-34, 09-54, 212, and 5007902 showed resistance to amoxicillin and ticarcillin but various levels of susceptibility to piperacillin, expanded-spectrum cephalosporins, and imipenem (Table 2). Addition of clavulanic acid and tazobactam did not restore the ß-lactam susceptibilities for amoxicillin and piperacillin, respectively (Table 2). Induction experiments with two *B. cenocepacia* strains (strains 212 and 5007902) showed a ca. 40-fold increase in the ß-lactamase activity by using imipenem as the inducer and cephalothin as the substrate. IEF analysis of cultures of all

TABLE 4. Amino acid identities between chromosome-encoded Pen-like proteins

Bacterial species	Pen homologue	% Amino acid sequence identity[a]											
		PenB1	PenA	PenC	PenD	PenE	PenF	PenG	PenH	PenI	PenJ	PenK	PenL
B. cenocepacia	PenB1		**82**	**92**	**91**	**85**	**83**	**84**	**73**	**69**	**64**	**63**	**71**
B. multivorans	PenA	**82**		84	85	85	82	84	78	63	64	64	66
B. stabilis	PenC	**92**	84		**86**	**85**	**82**	**78**	**76**	**62**	**62**	**61**	**66**
B. pyrrocina	PenD	**91**	85	**86**		**85**	**81**	**78**	**75**	**63**	**61**	**60**	**65**
B. vietnamiensis	PenE	**85**	85	**85**	**85**		**85**	**80**	**74**	**62**	**63**	**62**	**64**
B. ambifaria	PenF	**83**	82	**82**	**81**	**85**		83	75	59	59	60	62
B. dolosa	PenG	**84**	84	**78**	**78**	**80**	83		78	67	66	67	69
B. ubonensis	PenH	**73**	78	**76**	**75**	**74**	75	78		64	61	63	64
B. pseudomallei	PenI	**69**	63	**62**	**63**	**62**	59	67	64		83	98	89
B. oklahomensis	PenJ	**64**	64	**62**	**61**	**63**	59	66	61	83		82	87
B. mallei	PenK	**63**	64	**61**	**60**	**62**	60	67	63	98	82		88
B. thailandensis	PenL	**71**	66	**66**	**65**	**64**	62	69	64	89	87	88	

[a] The amino acid identities of the Pen-like proteins obtained in this study are indicated in boldface. The PenB1 (EU872211), PenC (FJ457906), PenD (FJ457907), and PenE (FJ386400) sequences were obtained from the strains used in this study (GenBank accession numbers are indicated in parentheses). PenA, PenF (YP_776246), PenG (EAY70884), PenH (ZP_02376965), PenI (YP_001062378), PenJ (ZP_02365625), PenK (YP_105905), and PenL (ZP_02384846) were obtained from the sequences deposited in the NCBI database (GenBank accession numbers are indicated in parentheses).

B. cenocepacia strains revealed a single ß-lactamase with activity and pI values that ranged from 7.5 to 8.5; the exception was strain 09-54, which coexpressed an additional ß-lactamase with a pI value of 6.0. This suggested the probable production of a similar ß-lactamase among those isolates.

Screening of PenA-like producers. Surprisingly, PCR assays with internal and external primers failed to identify a *penA*-like gene among the *B. cenocepacia* (belonging to genomovar groups I and III, together with *B. cepacia*), *B. vietnamiensis*, *B. pyrrocinia*, and *B. stabilis* isolates. However, positive results were obtained for the two *B. multivorans* strains. *B. multivorans* isolates 232 and 281 expressed ß-lactamases PenA2 and PenA3, which shared 96% and 99% amino acid sequence homologies with PenA (renamed PenA1), respectively (Table 3). These results agree with the reclassification of *B. cepacia* 249 (in which the *penA* gene had primarily been identified) as *B. multivorans* ATCC 17616 (36, 37).

Cloning of the *B. cenocepacia* 212 penicillinase-encoding gene. Cloning experiments were therefore performed to identify the naturally occurring ß-lactamase gene(s) of *B. cenocepacia.* Whole-cell DNA from *B. cenocepacia* isolate 212 was digested with Sau3AI and inserted in BK-CMV to give recombinant strain *E. coli* TOP10(pBcSau13). It expressed a penicillinase phenotype with resistance to amoxicillin and ticarcillin and reduced susceptibility to piperacillin and aztreonam. Addition of clavulanic acid and tazobactam only partially restored the ß-lactam activities of amoxicillin and piperacillin, respectively (Table 2). This phenotype agrees with the expression of a class A ß-lactamase with narrow-spectrum penicillinase activity. The recombinant strain remained susceptible to cephalosporins and imipenem, despite slight increases in their MICs (Table 2).

Identification of ß-lactamase PenB1. DNA sequence analysis of the 2,471-bp insert of pBcSau13 revealed two open reading frames that corresponded to β-lactamase PenB1 and a LysR-type regulator, PenR-B, respectively (Fig. 1). Two putative −10 and −35 promoter boxes were identified in the 140-bp intercistronic region extending between the two corresponding genes. Two potential ribosome-binding sites (RBSs) were also identified in that intergenic region; these were located 9 bp upstream of the *penB* gene and 8 bp upstream of the *penR-B* gene. Both RBS sequences were identical to those found upstream of the *penA* and *penR* genes in *B. multivorans* 249 (Fig. 1).

The *penB1* ß-lactamase gene identified was 936 bp and coded for a 312-amino-acid preprotein named PenB1 with a relative molecular mass of 32 kDa. The G+C content of this *penB1* gene was 69%, which is within the G+C content of the *B. cepacia* genome (66%). A search with the Signalp program (24) showed the presence of a signal peptide with a putative cleavage site between positions 33 and 34 of the N-terminal region. The resulting 279-amino-acid protein had a calculated molecular size of 29 kDa. PenB1 contains the four conserved motifs ^{70}S*XXK^{73} (where ^{70}S* is the active-site serine), ^{130}SDN^{133}, ^{166}EXXXN^{170}, and ^{234}KTG^{236} of class A ß-lactamases (by use of the numbering scheme of Ambler et al. [1]) (Fig. 1). Analysis of the insert sequence of recombinant plasmid pBsSau13 evidenced a gene encoding a putative LysR regulator that we named PenR-B, that is located upstream of *penB1*, and that is transcribed in an orientation opposite that of *penB1* (Fig. 1).

ß-Lactamase PenB1 shared 82% amino acid identity with PenA from *B. multivorans* 249 (formerly *B. cepacia*) (Table 4). In addition, ß-lactamase PenB1 shared 85, 84, 71, and 69% amino acid identities with putative class A ß-lactamases identified from the genomes of *Burkholderia vietnamiensis* strain 383, *Burkholderia dolosa* AUO158, *Burkholderia thailandensis* E264, and *Burkholderia pseudomallei* 668, respectively (in silico analysis). The closest amino acid identities with plasmid-mediated class A ß-lactamases were 59% and 51% with CTX-M-14 and CTX-M-2, respectively.

The PenR-B regulatory protein shared 97% amino acid identity with the LysR transcriptional regulator PenR from *Burkholderia* sp. strain 383 and 95% and 92% amino acid identities with the PenR proteins from *B. dolosa* AUO158 and *B. multivorans* 249, respectively. The PenR-B sequence also showed some identity with ß-lactamase transcriptional regulators of the LysR family, such as the AmpR proteins regulating the AmpC expression of *Pseudomonas aeruginosa* PAO1 (59%) and *Proteus vulgaris* B317 CumR (58.7%) (9, 19). A

TABLE 5. Kinetic parameters of purified PenB1 β-lactamase[a]

Substrate	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($mM^{-1} \cdot s^{-1}$)
Benzylpenicillin	130	25	5,200
Ampicillin	70	100	700
Ticarcillin	15	60	250
Piperacillin	5	10	500
Cephalothin	300	100	3,000
Cefuroxime	170	80	2,100
Cefoxitin	ND[b]	ND	ND
Cefotaxime	20	150	130
Ceftazidime	1.5	3,000	0.5
Cefepime	10	1,000	10
Aztreonam	30	2,500	10
Imipenem	0.1	60	2
Meropenem	1	2,500	0.5

[a] The data are the means of three independent experiments. Standard deviations were within 10% of the means.

[b] ND, no detectable hydrolysis (<0.01 s^{-1}) with a maximum amount of 5 μg of purified enzyme and up to 200 nmol of substrate.

search for a peptide motif, which was performed with the Genetics Computer Group program Motifs and the database Prosite, identified a helix-turn-helix motif in the N-terminal part of the PenR peptide sequence (^{25}FTRAGLELSVTQAA VSQQVRS^{45}), as is usually found for LysR-type transcriptional regulators.

Biochemical properties of PenB1. IEF analysis of cultures of *E. coli* TOP10(pBcSau13) revealed a pI value of 8.5. After purification, the specific activity of the PenB1 ß-lactamase against 100 μM benzylpenicillin was 520 U · mg of protein^{-1}, its purity was estimated to be >95% by SDS-PAGE analysis, and its purification coefficient was calculated to be 200. The kinetic parameters for the purified PenB1 ß-lactamase showed an hydrolysis profile that included penicillins, expanded-spectrum cephalosporins, and aztreonam. Imipenem and meropenem were hydrolyzed at low levels, whereas the hydrolysis of moxalactam and cefoxitin was not detected (Table 5).

Inhibition studies, as measured by determination of the IC_{50}s, showed that the activity of PenB1 was inhibited by tazobactam (0.5 μM) but that its activity was inhibited very poorly by clavulanic acid (10 μM), in accordance with what has been found for PenA, which was previously considered clavulanic acid resistant (27, 28).

Distribution of *penB1*-like genes among the *B. cepacia* complex. PCR screening was performed with internal and external primers designed from the *penB1* sequence and 11 strains belonging to the *B. cepacia* complex. A *penB1*-like gene was

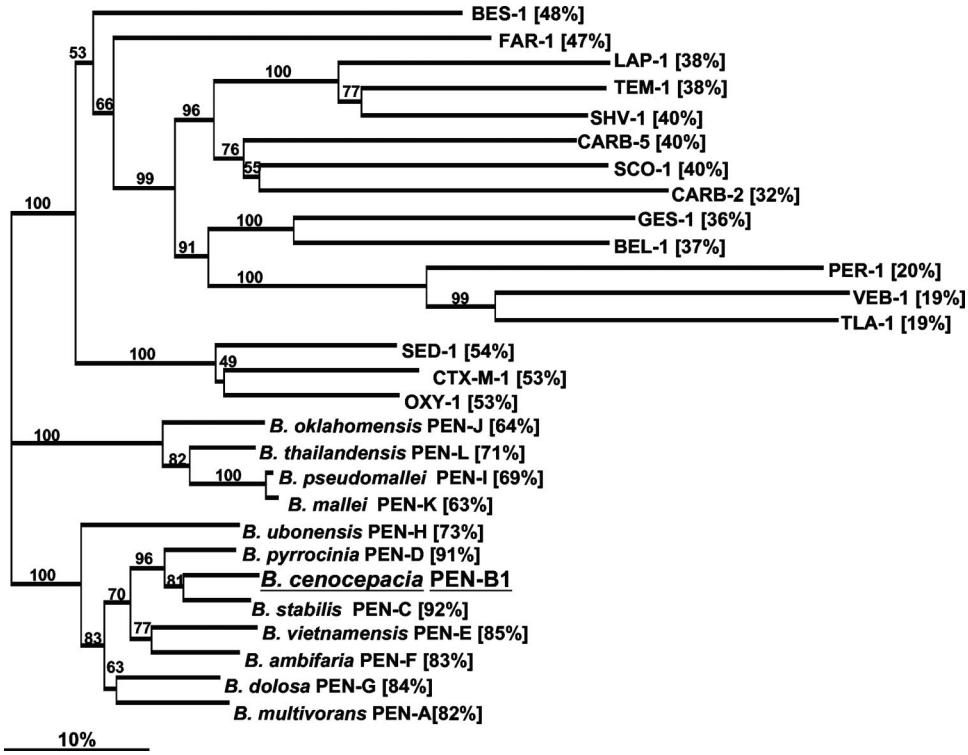

FIG. 2. Dendrogram obtained for representative class A ß-lactamases by neighbor-joining analysis. The alignment used for tree calculation was performed with the ClustalX program. Branch lengths are drawn to scale and are proportional to the number of amino acid changes. The distance along the vertical axis has no significance. The amino acid identity of each ß-lactamase compared to the amino acid sequence of PenB1 from *B. cenocepacia* is indicated in parentheses. The acquired ß-lactamases of gram-negative organisms (GenBank accession numbers are indicated in parentheses) are TEM-1 (AAG47772), SHV-1 (AAD18054), CTX-M-1 (CAJ01342), LAP-1 (ABK58097), CARB-2 (Q03170), CARB-5 (AAF61417), GES-1 (AAL82589), BEL-1 (AAZ04368), BES-1 (AAF61147), SCO-1 (ABL75133), PER-1 (CAF18433), and TLA-1 (AAD37403), whereas the others are naturally occurring ß-lactamases, such as FAR-1 from *Nocardia farcinica* (AAB81957), SED-1 from *Citrobacter sedlakii* (AAK63223), and OXY-1 from *Klebsiella oxytoca* (P22391).

identified in all those strains, making it a feature of the *B. cepacia* complex.

First, sequencing showed that the seven *B. cenocepacia* isolates contained PenB1-like-encoding sequences, namely, PenB2, PenB3, and PenB4, which had amino acid identities that ranged from 96 to 100% compared with the amino acid sequence of PenB1 (Table 3). Screening of the non-*B. cenocepacia* species identified *penB*-like genes encoding ß-lactamases and amino acid identities that ranged from 81 to 92% compared with the amino acid sequence of PenB1 (Table 4). Altogether, those results indicate that PenB1-like ß-lactamases are broadly distributed among isolates of the *B. cepacia* complex and may likely contribute to the natural ß-lactam resistance pattern observed.

Second, PCR screening of *penB*-like genes followed by sequencing revealed homologous sequences from the species *B. stabilis*, *B. pyrrocinia*, and *B. vietnamiensis*. Interestingly, *B. stabilis* 625 (genomovar IV) expressed ß-lactamase PenC1, which shared 92% amino acid identity with the amino acid sequence of PenB1 and 84% amino acid identity with the amino acid sequence of PenA1; *B. pyrrocinia* 685 (genomovar IX) expressed ß-lactamase PenD1, which shared 91% amino acid identity with the amino acid sequence of PenB1 and 85% amino acid identity with the amino acid sequence of PenA1; and *B. vietnamiensis* isolates 189 and 764 (genomovar V) expressed ß-lactamases PenE1 and PenE2 (three substitutions), respectively, and shared 85% amino acid identity with the amino acid sequences of both PenB1 and PenA1 (Table 4).

Conclusions. Here we showed that the *Burkholderia* complex possesses related but distinct, naturally occurring class A ß-lactamases very likely specific for each *Burkholderia* species. These findings are interesting because they may provide a useful tool for the identification of *Bulkholderia* isolates at the species level. We have characterized the PenB1 enzyme, which shares properties with the previously characterized PenA ß-lactamase from *B. multivorans*. PenB-like enzymes are naturally expressed by *B. cenocepacia*, which is the most prevalent *Burkholderia* species identified from CF patients not only in France (3) but also in Italy (4). We also identified other novel Pen-type ß-lactamases from a variety of *Burkholderia* sp. isolates: PenC from *B. stabilis*, PenD from *B. pyrrocinia*, and PenE from *B. vietnamiensis.* Finally, we took this opportunity to define a nomenclature for other *Burkholderia* spp. on the basis of an in silico analysis that makes PenF the class A ß-lactamase of *B. ambifira*, PenG that of *B. dolosa*, PenH that of *B. ubonensis*, PenI that of *B. pseudomallei*, PenJ that of *B. oklahomensis*, PenK that of *B. mallei*, and PenL that of *B. thailandensis* (Table 4; Fig. 2).

We showed that the expression of the *penB1* gene is inducible and is regulated by a LysR-type transcriptional regulator, as observed for *penA* from *B. multivorans*. This LysR-type dependence of the regulation of those class A ß-lactamases is similar to that observed with naturally occurring ß-lactamase genes from *Proteus vulgaris* (9), *Rhodopseudomonas capsulata* (7), and *Citrobacter diversus* (17) or with acquired bla_{SME}-like genes from *Serratia marcescens* (22), bla_{NMC-A} from *Enterobacter cloacae* (23),and bla_{IMI}-like genes from *E. cloacae* or *Enterobacter asburiae* (2, 29).

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