ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2005, p. 3743–3748 Vol. 49, No. 9
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.9.3743–3748.2005

BEL-1, a Novel Clavulanic Acid-Inhibited Extended-Spectrum β-Lactamase, and the Class 1 Integron In120 in *Pseudomonas aeruginosa*

Laurent Poirel,[1] Laura Brinas,[1,2] Annemie Verlinde,[3] Louis Ide,[3] and Patrice Nordmann[1]*

Service de Bactériologie-Virologie, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris-Sud, Université Paris XI, Le Kremlin-Bicêtre, France[1]; Area de Bioquimica y Biologia Molecular, Universidad de La Rioja, 26006 Logrono, Spain[2]; and Department of Microbiology, Heilig Hartziekenhuis Roeselare, Wilgenstraat 2, 8800 Roeselare, Belgium[3]

Received 16 April 2005/Returned for modification 31 May 2005/Accepted 1 July 2005

Screening by a double-disk synergy test identified a *Pseudomonas aeruginosa* isolate that produced a clavulanic acid-inhibited expanded-spectrum β-lactamase (ESBL). Cloning and sequencing identified a novel ESBL, BEL-1, weakly related to other Ambler class A ESBLs. β-Lactamase BEL-1 hydrolyzed significantly most expanded-spectrum cephalosporins and aztreonam, and its activity was inhibited by clavulanic acid, tazobactam, cefoxitin, moxalactam, and imipenem. This chromosome-encoded ESBL gene was embedded in a class 1 integron containing three other gene cassettes. In addition, this integron was bracketed by Tn*1404* transposon sequences at its right end and by *P. aeruginosa*-specific sequences at its left end.

Clavulanic acid-inhibited Ambler class A extended-spectrum β-lactamases (ESBLs) are rarely reported in *Pseudomonas aeruginosa*. To date, there have been five reports of TEM- and SHV-type ESBLs in *P. aeruginosa* (SHV-2a, SHV-5, TEM-4, TEM-24, and TEM-42), and three non-TEM-, non-SHV-type ESBLs have also been reported in that species (PER-1, VEB-like, and GES-like β-lactamases) (35). The $bla_{\text{PER-1}}$ β-lactamase gene is most widespread in Turkey (33), VEB-1 is most widespread in Southeast Asia (9), and GES-like β-lactamases have been identified in French, Greek, and South African isolates (8, 16, 26). Interestingly, GES-2, a point mutant derivative of GES-1 that was identified in South Africa, possessed an ability to hydrolyze not only expanded-spectrum cephalosporins but also imipenem (27).

The genetic vehicles that carry ESBL genes are variable. The bla_{TEM} genes are part of Tn*1*, Tn*2*, and Tn*3* transposon structures (19), bla_{SHV} genes are part of IS*26*-related transposon structures, and $bla_{\text{PER-1}}$ is part of a peculiar composite transposon, Tn*1213*, made of two different insertion sequence elements belonging to the IS*4* family (21). The bla_{VEB} and bla_{GES} genes are in the form of gene cassettes integrated into class 1 integrons (22, 24). In addition, a peculiar genetic organization involving 135-bp repeated elements (Re) bracketing a $bla_{\text{VEB-1a}}$ gene cassette has been recently identified in a *P. aeruginosa* isolate from India (2).

This study characterized a novel ESBL whose gene was identified in a class 1 integron.

MATERIALS AND METHODS

Bacterial strains. *P. aeruginosa* clinical isolate 51170 was identified with the API-20 NE system (bioMérieux, Marcy l'Etoile, France). *Escherichia coli* DH10B was the host for cloning experiments, and in vitro-obtained, rifampin-resistant *P. aeruginosa* PU21 was used as a recipient strain for transformation experiments (25).

Susceptibility testing. Antibiotic-containing disks were used for routine antibiograms by the disk diffusion assay (Sanofi-Diagnostic Pasteur, Marnes-la-Coquette, France) as previously described (20). The double-disk synergy test was performed with disks containing ceftazidime or cefepime and ticarcillin-clavulanic acid on Mueller-Hinton agar plates, and the results were interpreted as described previously (10). MICs were determined by an agar dilution technique with Mueller-Hinton agar (Sanofi-Diagnostic Pasteur) with an inoculum of 10^4 CFU per spot as described previously (10). All plates were incubated at 37°C for 18 h at ambient atmosphere. MICs of β-lactams were determined alone or in combination with fixed concentrations of clavulanic acid (2 μg/ml), tazobactam (4 μg/ml), and sulbactam (4 μg/ml). MIC results were interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards (17).

PCR and hybridization experiments. Total DNA of *P. aeruginosa* 51170 was extracted as described previously (20). This DNA was used as a template under standard PCR conditions (30) with a series of primers designed for detection of the following class A β-lactamase genes and their extended-spectrum derivatives found in enterobacterial and *P. aeruginosa* isolates: bla_{TEM}, bla_{SHV}, $bla_{\text{PER-1/2}}$, bla_{VEB}, and bla_{GES} (9, 22). Southern hybridizations were performed as described by Sambrook et al. (30) using the ECL nonradioactive labeling and detection kit (Amersham Pharmacia Biotech, Orsay, France).

Cloning experiments, recombinant plasmid analysis, and DNA sequencing. Total DNA of *P. aeruginosa* isolate 51170 was partially digested by the Sau3AI restriction enzyme, ligated into the BamHI site of plasmid pBK-CMV, and transformed into reference strain *E. coli* DH10B as previously described (20). Recombinant plasmids were selected on Trypticase soy agar plates containing amoxicillin (50 μg/ml) and kanamycin (30 μg/ml). The cloned DNA fragments of several recombinant plasmids, including pBEL-1, were sequenced on both strands with an Applied Biosystems sequencer (ABI 3100; Applied Biosystems, Foster City, Calif.). The entire sequence provided in this study was made of sequences of several plasmids that contained overlapping cloned fragments. The nucleotide and deduced amino acid sequences were analyzed and compared to sequences available over the Internet at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov).

Genetic support. Transformation experiments were performed with *P. aeruginosa* 51170 and in vitro-obtained, rifampin-resistant *P. aeruginosa* strain PU21 as previously described (25). Extraction of plasmid DNA from *P. aeruginosa* 51170 was attempted with the QIAGEN plasmid DNA maxi kit (QIAGEN, Courtaboeuf, France) and by the Kieser method (12). To search for a chromosomal location of the β-lactamase gene, we used the endonuclease I-CeuI (Amersham Phamacia Biotech) (14), which digests a 26-bp sequence in *rrn* genes for the 23S large-subunit rRNA, and separated the fragments by pulsed-field gel electrophoresis as previously described (23). Hybridization was performed with two

* Corresponding author. Mailing address: Service de Bactériologie-Virologie, Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre cedex, France. Phone: 33-1-45-21-36-32. Fax: 33-1-45-21-63-40. E-mail: nordmann.patrice@bct.ap-hop-paris.fr.

different probes, a 1,504-bp PCR-generated probe specific for 16S and 23S rRNA genes (9) and a 448-bp probe specific for the bla_{BEL-1} gene generated with internal primers BEL-A (5′-CGACAATGCCGCAGCTAACC-3′) and BEL-B (5′-CAGAAGCAATTAATAACGCCC-3′).

β-Lactamase purification and isoelectric focusing (IEF) analysis. Cultures of *E. coli* DH10B(pBEL-1) were grown overnight at 37°C in 4 liters of Trypticase soy broth containing amoxicillin (100 μg/ml) and kanamycin (30 μg/ml). β-Lactamase was purified by ion-exchange chromatography. Briefly, the β-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 β-lactamase-containing fractions were eluted with a linear 0 to 0.5 mM NaCl gradient. The fractions containing the highest β-lactamase activity were dialyzed against 20 mM Bis-Tris buffer (pH 5.5) and subsequently reloaded onto the preequilibrated Q-Sepharose column. The β-lactamase activity was recovered in the flowthrough, and then the extract was concentrated using an ultrafiltration filter tip (Sartorius, Göttingen, Germany). The purity of the enzyme was estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis (30).

IEF analysis was performed with an ampholine polyacrylamide gel (pH 3.5 to 9.5) as described previously (20), using a purified β-lactamase extract from a culture of *E. coli* DH10B(pBEL-1). The focused β-lactamases were detected by overlaying the gel with 1 mM nitrocefin (Oxoid, Dardilly, France) in 100 mM phosphate buffer (pH 7.0).

The putative location of the signal peptide cleavage site has been determined by using software available on the SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP/).

Kinetic measurements. Purified β-lactamase was used for kinetic measurements performed at 30°C with 100 mM sodium phosphate (pH 7.0) with an ULTROSPEC 2000 UV spectrophotometer (Amersham Pharmacia Biotech). Fifty percent inhibitory concentrations (IC_{50}s) were determined for clavulanic acid, tazobactam, sulbactam, cefoxitin, moxalactam, and imipenem. Various concentrations of these inhibitors were preincubated with the purified enzyme for 3 min at 30°C to determine the concentrations that reduced the hydrolysis rate of 100 μM benzylpenicillin by 50%.

The specific activity of the purified β-lactamase from *E. coli* DH10B(pBEL-1) was obtained as described previously (24). One unit of enzyme activity was defined as the activity which hydrolyzed 1 μmol of benzylpenicillin per min per mg of protein. The total protein content was measured with the DC protein assay kit (Bio-Rad, Ivry-sur-Seine, France).

Nucleotide sequence accession number. The nucleotide sequences reported in this work have been deposited in the GenBank nucleotide sequence database under accession no. DQ089809.

RESULTS

Properties of *P. aeruginosa* isolate 51170. *P. aeruginosa* isolate 51170 was isolated in May 2004 at the Heilig Hartziekenhuis, Roeselare, Belgium, from a scrotal swab of a 72-year-old man hospitalized for a dissecting aneurism of the left arteria renalis. He did not have any history of recent travel or hospitalization elsewhere. *P. aeruginosa* 51170 was resistant to ticarcillin and ceftazidime; had reduced susceptibility to piperacillin, cefepime, cefpirome, and aztreonam; and was susceptible to imipenem according to disk diffusion susceptibility testing. Double-disk synergy testing was positive with clavulanate-ceftazidime and clavulanate-aztreonam combinations (data not shown). *P. aeruginosa* 51170 was also resistant to aminoglycosides, fluoroquinolones, rifampin, chloramphenicol, and tetracycline and remained susceptible to fosfomycin and colistin.

Cloning and sequencing of the β-lactamase gene. Preliminary attempts to detect by PCR several Ambler class A ESBL-encoding genes failed (data not shown). Using total DNA of *P. aeruginosa* 51170 as a template in cloning experiments, several recombinant plasmids, including pBEL-1, were obtained. Sequence analysis of a ca. 10-kb cloned fragment of pBEL-1 revealed an 852-bp-long open reading frame encoding a 283-amino-acid preprotein. This protein was a β-lactamase designated BEL-1 (for Belgium extended β-lactamase) with the STFK, SDN, and KTG structural elements characteristic of the active site of Ambler class A β-lactamases (Fig. 1) (1, 15). The G+C content of bla_{BEL-1} was 48.4%, a value which differs significantly from the G+C content of *P. aeruginosa* genes (60.1 to 69.5%). BEL-1 was distantly related to other class A β-lactamases. Indeed, the highest percentages of amino acid identity were 50% with ESBL GES-1 and 40% with ESBLs CTX-M-2, CTX-M-3, and CTX-M-8. β-Lactamase BEL-1 shared 36% identity with ESBL BES-1, 35% with SHV-1, 34% with TEM-1, and only 21 and 19% with ESBLs PER-1 and VEB-1, respectively. The position of the leader peptide cleavage site would be between amino acids 20 and 21 (Ala and Asp, respectively) (Fig. 1).

β-Lactam susceptibility. MICs of β-lactams for *P. aeruginosa* 51170 and for *E. coli* DH10B(pBEL-1) indicated the expression of a clavulanic acid-inhibited β-lactamase that hydrolyzed expanded-spectrum cephalosporins but did not confer significant reduced susceptibility to carbapenems (Table 1).

Biochemical properties of BEL-1. IEF analysis showed that *P. aeruginosa* 51170 and *E. coli* DH10B(pBEL-1) had β-lactamase activities with a pI value of 7.1, corresponding to that of BEL-1 (data not shown). β-Lactamase activity with a pI value of 8.5 was also detected for *P. aeruginosa* 51170, corresponding to the chromosomal cephalosporinase of *P. aeruginosa*. The specific activity of the purified β-lactamase BEL-1 was 43 U mg of protein^{-1}. Its overall recovery was 80% with 70-fold purification. The purity of the enzyme was estimated to be more than 95% according to sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis (data not shown). Kinetic parameters of BEL-1 showed its broad-spectrum activity against most β-lactams, including expanded-spectrum cephalosporins and aztreonam but excluding cephamycins and carbapenems (Table 2). Analysis of the relative hydrolysis rates of BEL-1 showed that ceftriaxone was five- to sixfold more hydrolyzed than benzylpenicillin, and cefotaxime was also a good substrate. Although ceftazidime was well hydrolyzed, its kinetic parameters could not be determined precisely. This was due to a very high K_m value (>700 μM) reflecting a very low affinity of BEL-1 for ceftazidime, as is commonly observed with many ESBLs (4, 22, 24). IC_{50} determinations performed with benzylpenicillin as a substrate showed that BEL-1 activity was very well inhibited by clavulanic acid (0.1 μM) and exhibited lower susceptibility to sulbactam (2.2 μM) and tazobactam (3 μM). It was also, but to a lesser extent, inhibited by moxalactam (7 μM). In addition, BEL-1 activity was inhibited by imipenem (2 μM), as observed for VEB-1 and GES-1 (22), and by cefoxitin (9 μM), as observed for VEB-1 (24).

Genetic environment of bla_{BEL-1}. Sequence analysis of the inserts of several recombinant plasmids harboring the bla_{BEL-1} gene revealed that it was in the form of a gene cassette located in a class 1 integron structure named In120 (Fig. 2). The bla_{BEL-1} gene cassette has a perfect core site (GTTAGAC) and an inverse core site (GTCTAAC), and the length of its 59-be sequence is 63 bp, including nonperfect internal 2L (CCTA AGC) and 2R (GCTTGGC) sequences separated by 23 bp. This 59-be element did not show identity with any other known 59-be element.

Four gene cassettes were identified in In120, with the bla_{BEL-1}

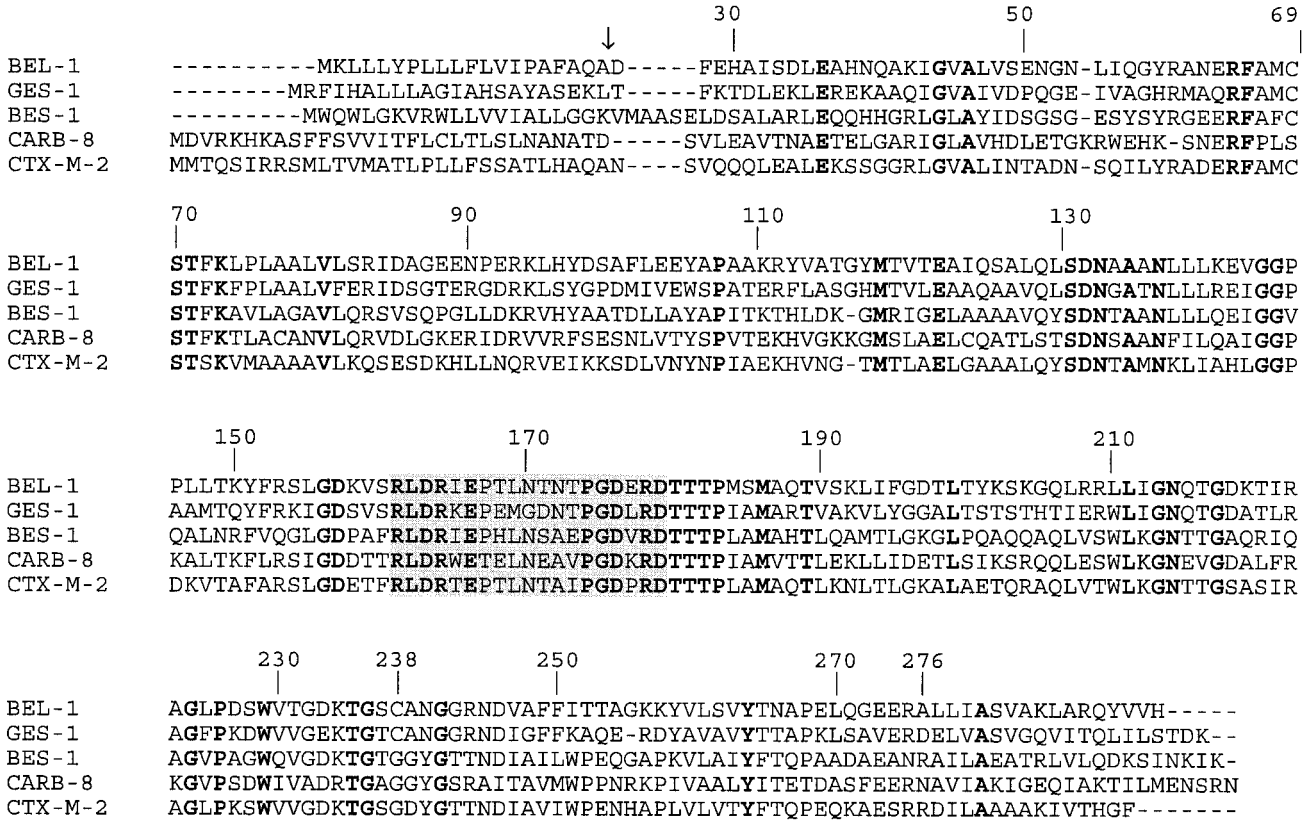

FIG. 1. Comparison of the amino acid sequence of β-lactamase BEL-1 to those of the most closely related ESBLs. Bolded amino acids are conserved residues. Numbering of β-lactamases is according to the nomenclature of Ambler et al. (1). The omega loop region is shaded in gray (3). The putative position of the leader peptide cleavage site for BEL-1 is indicated by a vertical arrow.

gene cassette being at the second position (Fig. 2). Upstream of bla_{BEL-1}, the *aacA4* aminoglycoside resistance gene encoding a 6′-*N*-acetyltransferase was found. Downstream of bla_{BEL-1}, a gene cassette encoding the putative small drug resistance protein SMR-2 was identified. This 105-amino-acid protein likely belongs to the SugE-type efflux protein subfamily known to confer resistance to toxic quaternary ammonium compounds (6). An identical gene cassette was identified recently in another integron together with the metallo-β-lactamase bla_{VIM-1} gene (32) and with a bla_{GES}-like positive isolate (34). Downstream of the *smr2* gene cassette, the *aadA5* gene cassette was identified that encodes an aminoglycoside-3′-adenylyltransferase conferring resistance to streptomycin and spectinomycin (36).

Analysis of the 5′-end sequence of the integron showed that the P_1 promoter sequences were located in the structural integrase gene, but no secondary (P_2) promoter was identified (7, 13). Thus, the gene cassettes located in that integron are under the control of weak promoter sequences.

Analysis of the right end of this integron showed that the 3′ conserved segment made of the *qacEΔ1*, *sul1*, and *orf5* genes was flanked by an incomplete set of *tni* genes as identified in In0, In2, In5, and In31 and considered defective derivatives of Tn*402*-like transposable elements (Fig. 2) (5). The *tniA* allele of the Tn*402*-tni module was flanked by the IRt end of the transposon (Fig. 2).

TABLE 1. MICs of β-lactams for *P. aeruginosa* clinical isolate 51170, *E. coli* DH10B harboring recombinant plasmid pBEL-1 expressing β-lactamase BEL-1, and reference strain *E. coli* DH10B

β-Lactam(s)[a]	MIC (μg/ml)		
	P. aeruginosa 51170	*E. coli* DH10B(pBEL-1)	*E. coli* DH10B
Amoxicillin	>512	>512	4
Amoxicillin + CLA	>512	512	4
Ticarcillin	>512	>512	4
Ticarcillin + CLA	128	64	4
Piperacillin	16	128	1
Piperacillin + TZB	8	32	1
Cephalothin	>512	512	4
Cefuroxime	>512	256	2
Ceftazidime	32	16	0.06
Ceftazidime + CLA	4	2	0.06
Ceftazidime + TZB	16	8	0.06
Ceftazidime + SUL	8	8	0.06
Cefotaxime	32	1	0.12
Ceftriaxone	256	16	0.12
Cefsulodin	>512	>512	16
Cefepime	4	0.25	0.06
Cefpirome	16	0.25	0.06
Cefoxitin	512	8	4
Moxalactam	512	0.12	0.06
Aztreonam	32	16	0.12
Aztreonam + CLA	4	4	0.12
Imipenem	1	0.12	0.06

[a] CLA, clavulanic acid at a fixed concentration of 2 μg/ml; TZB, tazobactam at a fixed concentration of 4 μg/ml; SUL, sulbactam at a fixed concentration of 4 μg/ml.

TABLE 2. Kinetic parameters of purified β-lactamase BEL-1[a]

Substrate	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($mM^{-1} \cdot s^{-1}$)	Relative k_{cat}/K_m (%)
Benzylpenicillin	3	20	150	100
Amoxicillin	10	30	330	220
Ticarcillin	6	8	750	500
Piperacillin	2	15	130	90
Cephalothin	150	280	540	360
Cephaloridine	30	130	230	150
Cefuroxime	10	40	250	160
Cefsulodine	25	150	170	110
Ceftazidime	>1.5	>700	nd[c]	nd
Cefotaxime	30	250	120	80
Ceftriaxone	25	30	820	550
Cefepime	1	150	7	5
Cefoxitin	<0.01	ND[b]		
Moxalactam	<0.01	ND		
Aztreonam	10	100	100	70
Imipenem	<0.01	ND		

[a] 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}).
[c] nd, not determinable due to too-low affinity.

Genetic environment of In120. Analysis of the 5′ end of In120 showed that an IS*Pa7* element was present 107 bp downstream of the *int1* integrase gene, as observed in integrons carrying the bla_{IMP-13} or the bla_{VIM-1} metallo-β-lactamase gene cassette identified in *P. aeruginosa* in Italy (29, 32) and in bla_{OXA-2}-containing integron In78 from *P. aeruginosa* from India (2). As found in the latter integrons, the transposase gene of IS*Pa7* was transcribed in the same orientation as that of the integrase gene (Fig. 2). IS*Pa7* is 1,669 bp long, possesses 17-bp inverted repeats, and belongs to the IS*NCY* family (http://www-is.biotoul.fr/). A same target site duplication made of GGCC was observed at both ends of IS*Pa7* that could suggest that this element had been integrated separately. However, analysis of the sequences present at the right end of IS*Pa7* did not identify specific sequences of the 5′ end of Tn*402*. Immediately upstream of the direct repeat generated by IS*Pa7* transposition, sequences very similar to that of *P. aeruginosa* strain UCBP-PA14 were identified (GenBank, unfinished genome sequence) (Fig. 2). Indeed, two open reading frames were identified, consisting of a 159-amino-acid putative protein-*S*-isoprenylcysteine methyltransferase (only one amino acid substitution) and a thioredoxin reductase (100% identity). Thus, it is likely that the sequences flanking the 5′ end of In120 corresponded to sequences of the chromosome of *P. aeruginosa* isolate 51170.

Analysis of the 3′ end of Tn*402*-borne integron In120 identified part of transposon Tn*1404* (18). Detailed analysis showed that In120-containing Tn*402* had been inserted inside the *res* resolution site of Tn*1404* (Fig. 2). This transposon had been identified in tetracycline-resistant *P. aeruginosa* isolates recovered from Michigan apple orchards (31). In the latter case, integration of a class 1 integron at the same location in the *res* site had been also observed. The sequences specific for Tn*1404*, particularly the *tnpA* gene encoding the transposase and the tetracycline resistance determinant, were not identified by PCR in *P. aeruginosa* 51170 (data not shown).

Genetic support of the ESBL determinant. Since no conjugation experiments were possible due to the lack of resistance markers available, direct transformation of putative plasmid DNA from *P. aeruginosa* 51170 to *P. aeruginosa* PU21 was

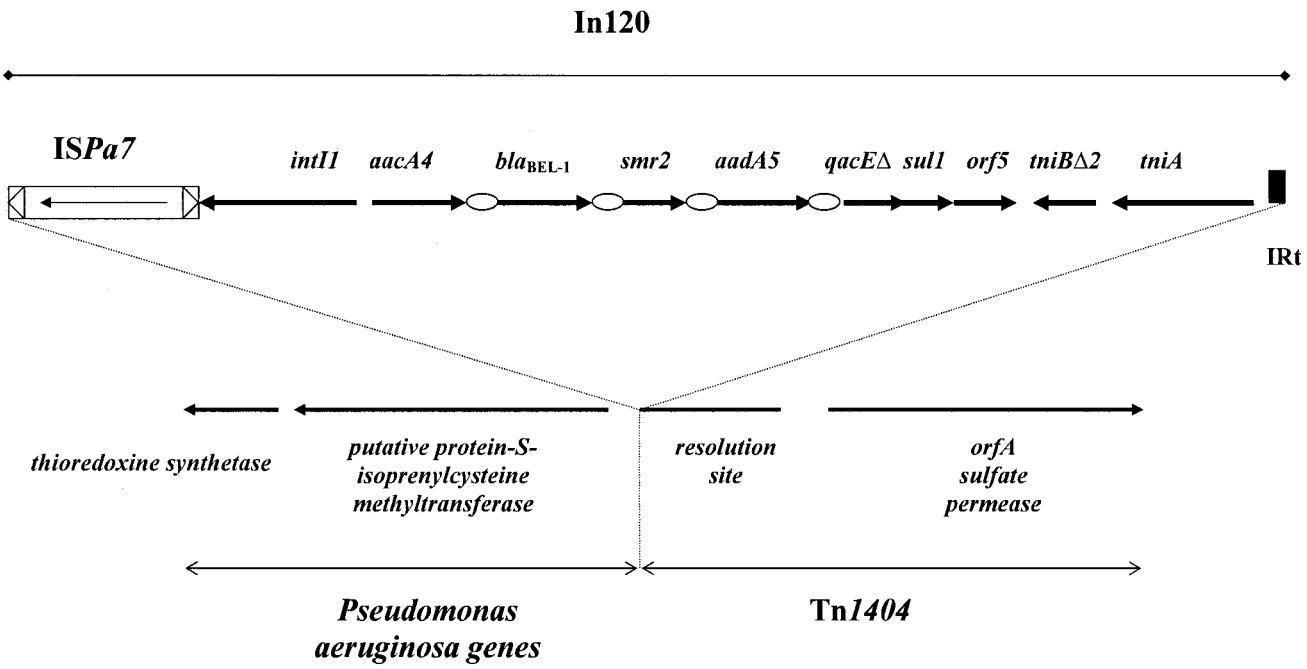

FIG. 2. Genetic environment of the bla_{BEL-1} gene. Open reading frames are indicated by horizontal arrows and 59-be elements by white circles. The inverted repeats of IS*Pa7* are indicated by triangles. The IRt end of In120 is indicated by a black vertical rectangle. This overall map is not to scale.

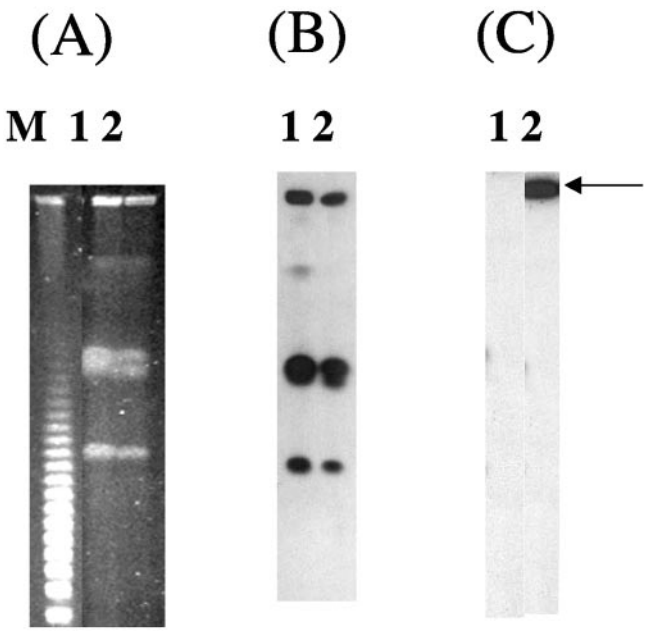

FIG. 3. (A) PFGE profiles of I-CeuI-digested whole-cell DNAs of reference strain *P. aeruginosa* PU21 (lane 1) and *P. aeruginosa* 51170 (lane 2). Lane M is for bacteriophage lambda concatemers used as molecular size markers (Bio-Rad). Southern hybridization performed with specific probes for the (B) 16S-23S rRNA gene of *P. aeruginosa* and for (C) the bla_{BEL-1} gene are shown. Horizontal arrow indicates position of hybridization with the bla_{BEL-1} probe.

attempted but remained unsuccessful. In addition, analysis of the plasmid content of *P. aeruginosa* 51170 did not identify any plasmid (data not shown), indicating a probable chromosomal location of the bla_{BEL-1} gene. Using the I-CeuI technique, we showed that the DNA probe for rRNA hybridized with all the fragments from the two *P. aeruginosa* strains, but hybridization with a DNA probe internal to the bla_{BEL-1} gene gave a single signal for *P. aeruginosa* 51170 only (Fig. 3). This signal corresponded to a large fragment that cohybridized with the rRNA probe, therefore corresponding to a chromosomal fragment. The latter result was in agreement with the identification of *P. aeruginosa*-specific sequences in the immediate vicinity of integron In120.

DISCUSSION

This report identified a novel ESBL that had weak amino acid identity with other ESBLs but had similar biochemical properties. β-Lactamase BEL-1 is a novel example of a non-TEM-, non-SHV-type ESBL in *P. aeruginosa*.

The relatively low-level MICs of expanded-spectrum β-lactams for the BEL-1-producing *P. aeruginosa* isolate may be explained in part by a lack of strong promoter sequences in the 5′-CS sequence of the bla_{BEL-1}-positive integron. This result strengthens the idea that ESBLs may be difficult to detect in *P. aeruginosa* and may therefore be clinically underestimated (35).

Compared to GES-1, BEL-1 shared most of the amino acids known to be critical for class A β-lactamases, such as Cys69, Glu104, Thr220, Cys238, and Ala240 (11). BEL-1 possesses a serine residue at position 237 that has been shown to be responsible for activity against expanded-spectrum cephalosporins. Indeed, in combination with some other residues, Ser237 is especially responsible for the hydrolysis of oxyimino β-lactams (15). As observed for GES-1, BEL-1 only slightly increases the MIC of cefotaxime once expressed in *E. coli*. However, the MIC of aztreonam is significantly increased when BEL-1 is expressed, which is not observed with GES-1.

BEL-1 was well inhibited by clavulanic acid, whereas its IC_{50} for tazobactam was 20-fold higher, indicating that tazobactam was a poor inhibitor of BEL-1 activity. BEL-1 is the second example of an ESBL that exhibits selective resistance to tazobactam after that of BES-1 (4). This property could be related to the presence of an alanine residue at position 276 replacing the usual arginine residue at that position in class A β-lactamases. The IC_{50} of imipenem for BEL-1 was similar to the values of other class A β-lactamases like GES-1 and VEB-1, underlining that inhibition by imipenem is a property shared at least by several class A β-lactamases, since it is not commonly evaluated. This property could be related to the presence of residues Cys69 and Cys238 forming a disulfide bridge and enhancing binding of imipenem, as observed for GES-1 and for carbapenem-hydrolyzing class A β-lactamases SME-1, NMC-A, and KPC-1. In addition, it is interesting that moxalactam was also a good inhibitor of BEL-1 activity, due to a high affinity of the enzyme for this substrate, as observed for TEM-52 (28).

Analysis of the surrounding sequences of bla_{BEL-1} showed that this gene was part of a gene cassette structure. The features of this mobile element entity were identified, including the core and inverse core sites, as well as the presence of a 59-be element. Interestingly, analysis of this overall structure indicated that acquisition of the resistance genes may have occurred by successive steps. It is likely that the primary Tn*1404* transposon structure had been truncated by the insertion of In120 since the right end of the integron was identified in the resolution site of Tn*1404*. Then, due to the fact that an IS*Pa7* element was present in the left end of In120 (as already observed for other integrons), the latter insertion sequence was at the origin of an integration process involving sequences located at its left end (Fig. 2). This In120-Tn*1404* structure was likely plasmid borne to enter the bacteria, and it targeted the chromosome of the *P. aeruginosa* recipient strain. Thus, this overall mechanism of acquisition likely corresponds to cointegration of a plasmid into the chromosome.

The bla_{BEL}, bla_{VEB}, and bla_{GES} β-lactamase genes encode class A ESBLs which all have been identified in *P. aeruginosa* and were located in class 1 integrons. It remains to evaluate whether the bla_{BEL-1} gene, as observed for the bla_{VEB} and bla_{GES} genes, has been disseminated among other gram-negative isolates, especially members of the family *Enterobacteriaceae*. This report underlines the variety of ESBL genes in *P. aeruginosa*, most of them being integron encoded and all of them still from unknown reservoirs. We believe that in many cases the environment-borne *P. aeruginosa* species may capture antibiotic resistance genes from other environmental species before exchanging those genes with members of the family *Enterobacteriaceae*.

ACKNOWLEDGMENTS

This work was funded by a grant from the Ministère de l'Education Nationale et de la Recherche (UPRES-EA3539), Université Paris XI, Paris, France, and by the European Community (6th PCRD, LSHM-CT-2003-503-335). L.P. is a researcher from the INSERM, Paris, France.

We thank C. Torres for constant interest in the work of L.B.

REFERENCES

1. **Ambler, R. P., A. F. Coulson, J.-M. Frère, J. M. Ghuysen, B. Joris, M. Forsman, R. C. Levesque, G. Tiraby, and S. G. Waley.** 1991. A standard numbering scheme for the class A β-lactamases. Biochem. J. **276:**269–270.
2. **Aubert, D., D. Girlich, T. Naas, S. Nagarajan, and P. Nordmann.** 2004. Functional and structural characterization of the genetic environment of an extended-spectrum β-lactamase bla_{VEB} gene from a *Pseudomonas aeruginosa* isolate obtained in India. Antimicrob. Agents Chemother. **48:**3284–3290.
3. **Banerjee, S., U. Pieper, G. Kapadia, L. K. Pannell, and O. Herzberg.** 1998. Role of the omega-loop in the activity, substrate specificity, and structure of class A β-lactamase. Biochemistry **37:**3286–3296.
4. **Bonnet, R., J. L. Sampaio, C. Chanal, D. Sirot, C. De Champs, J. L. Viallard, R. Labia, and J. Sirot.** 2000. A novel class A extended-spectrum β-lactamase (BES-1) in *Serratia marcescens* isolated in Brazil. Antimicrob. Agents Chemother. **44:**3061–3068.
5. **Brown, H. J., H. W. Stokes, and R. M. Hall.** 1996. The integrons In0, In2, and In5 are defective transposon derivatives. J. Bacteriol. **178:**4429–4437.
6. **Chung, Y. J., and M. H. Saier.** 2002. Overexpression of the *Escherichia coli sugE* gene confers resistance to a narrow range of quaternary ammonium compounds. J. Bacteriol. **184:**2543–2545.
7. **Collis, C. M., and R. M. Hall.** 1995. Expression of antibiotic resistance genes in the integrated cassettes of integrons. Antimicrob. Agents Chemother. **39:**155–162.
8. **Dubois, V., L. Poirel, C. Marie, C. Arpin, P. Nordmann, and C. Quentin.** 2002. Molecular characterization of a novel class 1 integron containing bla_{GES-1} and a fused product of *aac3-Ib*/*aac6′-Ib′* gene cassettes in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **46:**638–645.
9. **Girlich, D., T. Naas, A. Leelaporn, L. Poirel, M. Fennewald, and P. Nordmann.** 2002. Nosocomial spread of the integron-located *veb-1*-like cassette encoding an extended-spectrum β-lactamase in *Pseudomonas aeruginosa* in Thailand. Clin. Infect. Dis. **34:**175–182.
10. **Jarlier, V., M.-H. Nicolas, G. Fournier, and A. Philippon.** 1988. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. **10:**867–878.
11. **Joris, B., P. Ledent, O. Dideberg, E. Fonze, J. Lamotte-Brasseur, J. A. Kelly, J. M. Ghuysen, and J.-M. Frère.** 1991. Comparison of the sequences of class A β-lactamases and of the secondary structure elements of penicillin-recognizing proteins. Antimicrob. Agents Chemother. **35:**2294–2301.
12. **Kieser, T.** 1984. Factors affecting the isolation of CCC DNA from *Streptomyces lividans* and *Escherichia coli*. Plasmid **12:**19–36.
13. **Levesque, C., S. Brassard, J. Lapointe, and P. H. Roy.** 1994. Diversity and relative strength of tandem promoters for the antibiotic-resistance genes of several integrons. Gene **142:**49–54.
14. **Liu, S. L., A. Hessel, and K. E. Sanderson.** 1993. Genomic mapping with I-*Ceu*-I, an intron-encoded endonuclease specific for genes for ribosomal RNA in *Salmonella* spp., *Escherichia coli*, and other bacteria. Proc. Natl. Acad. Sci. USA **90:**6874–6878.
15. **Matagne, A., J. Lamotte-Brasseur, and J.-M. Frère.** 1998. Catalytic properties of class A β-lactamases: efficiency and diversity. Biochem. J. **330:**581–598.
16. **Mavroidi, A., E. Tzelepi, A. Tsakris, V. Miriagou, D. Sofianou, and L. S. Tzouvelekis.** 2001. An integron-associated β-lactamase (IBC-2) from *Pseudomonas aeruginosa* is a variant of the extended-spectrum β-lactamase IBC-1. J. Antimicrob. Chemother. **48:**627–630.
17. **National Committee for Clinical Laboratory Standards.** 2004. Performance standards for antimicrobial susceptibility testing. Eleventh informational supplement. M100-S14. National Committee for Clinical Laboratory Standards, Wayne, Pa.
18. **Partridge, S. R., C. M. Collis, and R. M. Hall.** 2002. Class 1 integron containing a new gene cassette, *aadA10*, associated with Tn*1404* from R151. Antimicrob. Agents Chemother. **46:**2400–2408.
19. **Partridge, S. R., and R. M. Hall.** 2005. Evolution of transposons containing bla_{TEM} genes. Antimicrob. Agents Chemother. **49:**1267–1268.
20. **Philippon, L. N., T. Naas, A.-T. Bouthors, V. Barakett, and P. Nordmann.** 1997. OXA-18, a class D clavulanic acid-inhibited extended-spectrum β-lactamase from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **41:**2188–2195.
21. **Poirel, L., L. Cabanne, H. Vahaboglu, and P. Nordmann.** 2005. Genetic environment and expression of the extended-spectrum β-lactamase bla_{PER-1} gene in gram negatives. Antimicrob. Agents Chemother. **49:**1708–1713.
22. **Poirel, L., I. Le Thomas, T. Naas, A. Karim, and P. Nordmann.** 2000. Biochemical sequence analyses of GES-1, a novel class A extended-spectrum β-lactamase, and the class 1 integron In52 from *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **44:**622–632.
23. **Poirel, L., O. Menuteau, N. Agoli, C. Cattoen, and P. Nordmann.** 2003. Outbreak of extended-spectrum β-lactamase VEB-1-producing isolates of *Acinetobacter baumannii* in a French hospital. J. Clin. Microbiol. **41:**3542–3547.
24. **Poirel, L., T. Naas, M. Guibert, E. B. Chaibi, R. Labia, and P. Nordmann.** 1999. Molecular and biochemical characterization of VEB-1, a novel class A extended-spectrum β-lactamase encoded by an *Escherichia coli* integron gene. Antimicrob. Agents Chemother. **43:**573–581.
25. **Poirel, L., T. Naas, D. Nicolas, L. Collet, S. Bellais, J. D. Cavallo, and P. Nordmann.** 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob. Agents Chemother. **44:**891–897.
26. **Poirel, L., G. F. Weldhagen, C. De Champs, and P. Nordmann.** 2002. A nosocomial outbreak of *Pseudomonas aeruginosa* isolates expressing the extended-spectrum β-lactamase GES-2 in South Africa. J. Antimicrob. Chemother. **49:**561–565.
27. **Poirel, L., G. F. Weldhagen, T. Naas, C. De Champs, M. G. Dove, and P. Nordmann.** 2001. GES-2, a class A β-lactamase from *Pseudomonas aeruginosa* with increased hydrolysis of imipenem. Antimicrob. Agents Chemother. **45:**2598–2603.
28. **Poyart, C., P. Mugnier, G. Quesne, P. Berche, and P. Trieu-Cuot.** 1998. A novel extended-spectrum TEM-type β-lactamase (TEM-52) associated with decreased susceptibility to moxalactam in *Klebsiella pneumoniae*. J. Clin. Microbiol. **36:**41–47.
29. **Riccio, M. L., L. Pallecchi, J. D. Docquier, S. Cresti, M. R. Catania, L. Pagani, C. Lagatolla, G. Cornaglia, R. Fontana, and G. M. Rossolini.** 2005. Clonal relatedness and conserved integron structures in epidemiologically unrelated *Pseudomonas aeruginosa* strains producing the VIM-1 metallo-β-lactamase from different Italian hospitals. Antimicrob. Agents Chemother. **49:**104–110.
30. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
31. **Schnabel, E. L., and A. L. Jones.** 1999. Distribution of tetracycline resistance genes and transposons among phylloplane bacteria in Michigan apple orchards. Appl. Environ. Microbiol. **65:**4898–4907.
32. **Toleman, M. A., D. Biedenbach, D. M. C. Bennett, R. N. Jones, and T. R. Walsh.** 2005. Italian metallo-β-lactamases: a national problem? Report from the SENTRY Antimicrobial Surveillance Programme. J. Antimicrob. Chemother. **55:**61–70.
33. **Vahaboglu, H., R. Öztürk, G. Aygun, F. Coskunkan, A. Yaman, A. Kaygusuz, H. Leblebicioglu, I. Balik, K. Aydin, and M. Otkun.** 1997. Widespread detection of PER-1-type extended-spectrum β-lactamases among nosocomial *Acinetobacter* and *Pseudomonas aeruginosa* isolates in Turkey: a nationwide multicenter study. Antimicrob. Agents Chemother. **41:**2265–2269.
34. **Vourli, S., P. Giakkoupi, V. Miriagou, E. Tzelepi, A. C. Vatopoulos, and L. S. Tzouvelekis.** 2004. Novel GES/IBC extended-spectrum β-lactamase variants with carbapenemase activity in clinical enterobacteria. FEMS Microbiol. Lett. **234:**209–213.
35. **Weldhagen, G. F., L. Poirel, and P. Nordmann.** 2003. Ambler class A extended-spectrum β-lactamases in *Pseudomonas aeruginosa*: novel developments and clinical impact. Antimicrob. Agents Chemother. **47:**2385–2392.
36. **White, P. A., C. J. McIver, Y. Deng, and W. D. Rawlinson.** 2000. Characterisation of two new gene cassettes, aadA5 and *dfrA17*. FEMS Microbiol. Lett. **182:**265–269.