ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2002, p. 1132–1135 Vol. 46, No. 4
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.4.1132–1135.2002

Cloning and Expression of Class A β-Lactamase Gene *blaA*$_{BPS}$ in *Burkholderia pseudomallei*

Terence K. M. Cheung, P. L. Ho,* Patrick C. Y. Woo, K. Y. Yuen, and P. Y. Chau

Department of Microbiology, Queen Mary Hospital, The University of Hong Kong, Hong Kong, China

Received 7 June 2001/Returned for modification 29 September 2001/Accepted 13 January 2002

The β-lactamase gene *blaA*$_{BPS}$ in *Burkholderia pseudomallei* was cloned and expressed in *Escherichia coli*. BPS-1 is a cephalosporinase with an isoelectric point of 7.7. Sequence analysis of BPS-1 revealed conserved motifs typical of class A β-lactamases and a relationship to the PenA (in *B. cepacia*) and BlaI (in *Yersinia enterocolitica*) lineages.

Burkholderia pseudomallei, the bacterium that causes melioidosis, is resistant to ampicillin and broad- and expanded-spectrum cephalosporins due to the production of a β-lactamase. The enzyme hydrolyzes some cephalosporins, such as cephalothin, cefuroxime, and cefotaxime, strongly (6). It behaves like the chromosomal cefuroximase of *Proteus vulgaris* and hence should be classified in the 2e β-lactamase group although it was placed in the 2be β-lactamase group earlier (2). Currently, the enzyme-resistant β-lactams, such as ceftazidime and β-lactam–β-lactamase inhibitor combinations, are the mainstays of treatment for all forms of melioidosis. A greater understanding of the β-lactamase in this organism is therefore desirable. In this paper, we report on the cloning and expression of a *B. pseudomallei* gene (*blaA*$_{BPS}$) that encodes the class A β-lactamase named BPS-1. The β-lactamase genes in a group of human, animal, and environmental isolates were sequenced and compared.

Seventeen strains of *B. pseudomallei* isolated from human, animal and environmental sources in Hong Kong and Thailand between 1975 and 1986 were studied. These isolates represent part of a collection that has been described earlier (4, 6, 13). Five human isolates were from Thailand. The remaining 12 isolates were from Hong Kong (5 from humans, 6 from deceased marine mammals, and 1 from soil). All strains were arabinose negative, and none were *B. thailendensis*.

The MICs of the β-lactam antibiotics were determined by the Etest (AB Biodisk, Solna, Sweden). β-Lactamase crude cell extracts were prepared from 100-ml cultures in Luria-Bertani broth (Oxoid, Basingstoke, United Kingdom) by using procedures described previously (6). In brief, cells were killed with toluene and disrupted by sonication. Enzyme yield was increased by a single extraction with Triton X-100 as previously described (6). Cellular debris was removed by centrifugation (30,000 × *g*), and the supernatant was collected as the source of β-lactamase. Enzyme extracts were frozen at −70°C until they were tested. Isoelectric focusing of the β-lactamases was performed with ampholine gel (Pharmacia, Hong Kong, China) from pI 3.5 to pI 9.5. Enzyme extracts from strains expressing TEM-1 (pI 5.4), OXA-1 (pI 7.4), and SHV-1 (pI 7.6) were used as controls. The pI value of each enzyme was determined by overlaying the gel with nitrocefin (12).

Crude lysate without purification was used for substrate and inhibition assays. The assays were performed spectrophotometrically by measuring the change in absorbance at the appropriate wavelength for each substrate by using a Beckman DU650 spectrophotometer (Beckman Coulter, Fullerton, Calif.) (6, 11). Hydrolysis assays were carried out in 0.5 ml of 10 mM phosphate buffer (AMRESCO Inc., Solon, Ohio), pH 7.4, at 37°C. Antibiotic powders with known potencies, including sulbactam (Pfizer Corporation, Hong Kong, China) and clavulanic acid (SmithKline Beecham, Hong Kong, China) were kindly provided by the manufacturers. All other antibiotics were purchased from Sigma (St. Louis, Mo.). The wavelengths were 235 nm for benzylpenicillin and ampicillin, 255 nm for cephaloridine and cephalothin, 257 nm for cefotaxime and ceftazidime, 263 nm for cloxacillin, and 274 nm for cefuroxime. V_{max} and K_m were calculated by the Lineweaver-Burk plot using the built-in Enzymatic Mechanism software (3). All kinetic studies were performed three times.

Cloning and sequence analysis of the β-lactamase gene from a *B. pseudomallei* strain (HK-PPM-1, a strain isolated from a dolphin in 1975) was performed as follows (9). A set of primers was used to amplify the β-lactamase gene from the genomic DNA. Primers were designed by a BLAST analysis of the *B. pseudomallei* genome sequence at the Sanger Center with β-lactamase gene *penA* from *B. cepacia*. The oligonucleotide primers used were forward primer F1 (5′-CCG GAA TTC ATG AAT CAT TCT CCG TTG CGC CGC TCG C-3′; nucleotide positions 1 to 28) and backward primer B1 (5′-GCT CTA GAT CAG GCG AAC GCC CGC GCG GCG ATC CGC-3′; nucleotide positions 888 to 861). Restriction sites (underlined) were incorporated into the primers (an *Eco*RI restriction site for the forward primer and an *Xba*I restriction site for the backward primer) for ligation of the amplified products to phagemid vector pBK-CMV (Kan^r; Stratagene, La Jolla, Calif.). Afterward, $CaCl_2$-treated *Escherichia coli* XL1-Blue MRF′ (ampicillin sensitive) was transformed with the recombinant plasmid (12) and clones were selected on Luria-Bertani agar supplemented with ampicillin (25 μg/ml) and kanamycin (50 μg/ml). The 0.9-kb DNA insert into the recombinant plasmids (pBKCMV01 and pBKCMV07) of two ampi-

* Corresponding author. Mailing address: Dept. of Microbiology, University Pathology Building, Queen Mary Hospital, The University of Hong Kong, Pokfulam Rd., Pokfulam, Hong Kong, China. Phone: (852) 2855 4892. Fax: (852) 2855 1241. E-mail: plho@hkucc.hku.hk.

TABLE 1. MICs of β-lactams for reference and β-lactamase (BPS-1)-producing strains

Drug	MIC (μg/ml)		
	B. pseudomallei PPM-1[a]	*E. coli* XL1-Blue MRF′[b]	*E. coli* P1 and P7[c]
Ampicillin	>32	4	>32
Amoxicillin-clavulanate	4	8	8
Cefuroxime	32	4	16 (P7), 64 (P1)
Cefotaxime	16	0.12	1
Ceftazidime	2	0.5	1
Cefepime	16	0.064	0.5
Cefpirome	16	0.12	1
Imipenem	2	0.25	0.25
Meropenem	2	0.016	0.032

[a] Parental *B. pseudomallei* strain HK-PPM-1 that produces BPS-1.
[b] Reference strain *E. coli* XL1-Blue MRF′ used for expression of BPS-1.
[c] *E. coli* XL1-Blue MRF′ strains (P1 and P7) transformed with recombinant plasmids pBK-CMV01 and pBK-CMV07 encoding $blaA_{BPS}$, respectively.

cillin-kanamycin-resistant clones (*E. coli* XL1-Blue MRF′ P1 and P7) was confirmed by sequencing using the T3 and T7 vector sequence as the primers. Bidirectional sequencing of both strands was performed by the Bigdye dideoxynucleotide chain termination method with an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer Corp., Foster City, Calif.). Subsequently, the β-lactamase-encoding genes in all of the strains were amplified and sequenced by using primers with the sequences 5′-ATC CGC CTG ATG AAT CAT TC-3′ (F2 forward, positions −9 to 11) and 5′-GCG CGC TCA GGC GAA CGC CC-3′ (B2 backward, positions 894 to 875). Around 600 to 650 bases of both strands were sequenced in each direction. Both strands of the β-lactamase-encoding genes in PPM-1, P1, and P7 were sequenced twice. Both strands of the β-lactamase gene variants were also sequenced twice. The β-lactamase sequences in the remaining strains were sequenced once.

Isoelectric focusing analysis of the parental strain (HK-PPM-1) and the two *E. coli* clones (P1 and P7) revealed only a single β-lactamase with the same isoelectric point of 7.7. The β-lactam susceptibility profiles of the parental *B. pseudomallei* strain (HK-PPM-1), the reference strain (*E. coli* XL1-Blue MRF′), and *E. coli* strains P1 and P7 are shown in Table 1. The kinetic parameters of the β-lactamase enzyme with a pI of 7.7 obtained from the *B. pseudomallei* parental strain and *E. coli* clone P1 are shown in Table 2.

Analysis of the DNA inserts in pBKCMV01 and pBKCMV07 showed an 888-nucleotide open reading frame that encodes a 295-amino-acid polypeptide. A search with the program Signalp (8) showed the presence of a signal peptide with a putative cleavage site at position 22 of the N-terminal region. For the putative mature protein of 274 amino acid residues, the calculated molecular size was 29.1 kDa. A comparison of the deduced amino acid sequence of this protein with those of known β-lactamases revealed a close relationship to the class A β-lactamases (Fig. 1). The following β-lactamases were found to have the highest degree of identity with the mature protein: PenA from *B. cepacia* (65%), BlaI from *Yersinia enterocolitica* (57.1%), CTX-M-18 from *Klebsiella pneumoniae* (55.9%), SFO-1 from *Enterobacter cloacae* (54.3%), FONTA-4 from *Serratia fonticola* (53.9%), and OXY from *Klebsiella oxytoca* (53.3%). BPS-1 contains the four conserved motifs, ^{70}SXXK^{73}, ^{130}SDN^{132}, ^{166}EXXXN^{170}, and ^{234}KTG^{236} (Ambler's numbering scheme), that are characteristics of class A β-lactamases (1). The sequences of the $blaA_{BPS-1}$ genes of the other 16 isolates were either identical to that of HK-PPM-1 or differed from it by only a few bases. These variations resulted in the following amino acid substitutions: Ile→Met at position 139 (1 strain, BPS-1b), Pro→Leu at position 145 (1 strain, BPS-1c), and Ala→Thr at position 147 (10 strains, BPS-1d). These substitutions had no correlation with the geographic source and host origin of the isolates.

The pI 7.7 β-lactamase, named BPS-1, had properties similar to those described previously for the β-lactamase of *B. pseudomallei* in several aspects (6). Firstly, the enzyme represents a cephalosporinase with strong activities against cephalothin, cephaloridine, cefuroxime, and cefotaxime. Secondly, this β-lactamase was inhibited by a low concentration of clavulanic

TABLE 2. Kinetic parameters of BPS-1 β-lactamase

Substrate or inhibitor	*B. pseudomallei* PPM-1[a]			*E. coli* P1[d]		
	Relative V_{max}[b]	K_m (μM)	IC_{50} (nM)[c]	Relative V_{max}	K_m (μM)	IC_{50} (nM)
Substrate						
Penicillin	100 ± 1[e]	42 ± 3		100 ± 0	36 ± 4	
Ampicillin	43 ± 9	30 ± 6		24 ± 8	32 ± 5	
Cephaloridine	140 ± 11	55 ± 2		110 ± 8	25 ± 2	
Cephalothin	480 ± 23	54 ± 3		410 ± 24	35 ± 3	
Cefuroxime	430 ± 53	170 ± 18		380 ± 38	230 ± 22	
Cefotaxime	120 ± 9	140 ± 15		110 ± 5	150 ± 10	
Ceftazidime	5.0			1.5		
Cloxacillin	0.4			0.1		
Inhibitor						
Clavulanate			46 ± 11			21 ± 4
Sulbactam			690 ± 220			410 ± 93

[a] Parental *B. pseudomallei* strain that produces BPS-1.
[b] Relative to that of penicillin which, was taken as 100%.
[c] IC_{50}, concentration of β-lactamase inhibitor that inhibits 50% of the activity after 10 min of incubation at 25°C. Cephalothin IC_{50}, (50 μM.) was used as the substrate.
[d] An *E. coli* XL1-Blue MR clone containing plasmid vector pBK-CMV01 with $blaA_{BPS}$.
[e] Data shown are the mean ± standard deviation of three measurements.

FIG. 1. Sequence alignment of the BPS-1 β-lactamase from *B. pseudomallei* with its nearest class A neighbors. Identical residues are in boldface. Colons were introduced to adjust the alignment. The start of the putative mature protein is underlined. The conserved motifs (^{70}SXXK^{73}, ^{130}SDN^{132}, ^{166}EXXXN^{170}, and ^{234}KTG^{236}) typical of class A β-lactamases are shaded. Arrows indicate the putative omega loop region (7). The ABL consensus sequence is shown for comparison (1). The sources (GenBank accession numbers) of the β-lactamases are as follows: BPS-1, *B. pseudomallei* (AF326770); PenA, *B. cepacia* (AAB53622); BlaI, *Y. enterocolitica* (CAA40357); CTX-M-13, *K. pneumoniae* (AAK55533); SFO-1, *E. cloacae* (BAA76882); FONA-4, *S. fonticola* (CAB61641); OXY-1a, *K. oxytoca* (CAB42615).

acid and less strongly by sulbactam. Furthermore, the molecular size of mature BPS-1 is quite similar to those of a serine β-lactamase previously purified from a *B. pseudomallei* clinical isolate, HK21 (6). However, the ability of BPS-1 from PPM-1 to hydrolyze both cefuroxime and cefotaxime was lower than that of the enzyme from HK21 described previously. This could be the result of testing of a different variant of the β-lactamase. On the basis of the gene sequence, at least three variants (BPS-1b, -1c, and -1d) can be identified although the effect of the amino acid substitution on the hydrolytic profile remains to be defined.

In terms of sequence homology, several lineages of class A β-lactamases can be distinguished (7). In this regard, BPS-1 is phylogenetically nearest to PenA of *B. cepacia* (14) and BlaI of *Y. enterocolitica* (10). A feature common to the three enzymes is that they are chromosomally encoded. Interestingly, BPS-1 is a cephalosporinase while the latter two are penicillinases. Despite these differences, the efficiencies of hydrolysis (V_{max}/K_m relative to that of ampicillin) of BPS-1 and PenA (5) for cefuroxime (176 and 188, respectively) and cefotaxime (60 and 57, respectively) are remarkably similar. Like BPS-1, BlaI of *Y. enterocolitica* is also a group 2e cefuroxime-hydrolyzing enzyme similar to the inducible cephalosporinases from *P. vulgaris* (2). Furthermore, BPS-1 also behaves like the related family of CTX-M β-lactamases that are endemic to Latin America and some areas of northeastern Europe (15). Like BPS-1, these enzymes hydrolyze cefotaxime but exhibit little activity against ceftazidime. Characteristically, enzymatic activities are inhibited by low concentrations of clavulanic acid and sulbactam. Unlike BPS-1, the CTX-M enzymes are encoded on plasmids.

In summary, the sequences of the class A enzyme BPS-1 in *B. pseudomallei* and other, related β-lactamases were compared. The sequences of the wild-type enzymes from human, animal, and soil isolates appeared to be quite homogeneous.

Nucleotide sequence accession numbers. The sequences of the $blaA_{BPS-1}$ genes have been given GenBank accession numbers AF326770 and AF441237 to -39.

This work was supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region (project HKU 7282/97 M).

REFERENCES

1. **Ambler, R. P., A. F. Coulson, J. M. Frere, 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 beta-lactamases. Biochem. J. **276:**269–270.
2. **Bush, K., G. A. Jacoby, and A. A. Medeiros.** 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. **39:**1211–1233.
3. **Bush, K., and R. B. Sykes.** 1986. Methodology for the study of β-lactamases. Antimicrob. Agents Chemother. **30:**6–10.
4. **Chau, P. Y., W. S. Ng, Y. K. Leung, and S. Lolekha.** 1986. In vitro susceptibility of strains of *Pseudomonas pseudomallei* isolated in Thailand and Hong Kong to some newer beta-lactam antibiotics and quinolone derivatives. J. Infect. Dis. **153:**167–170.
5. **Hirai, K., S. Iyobe, M. Inoue, and S. Mitsuhashi.** 1980. Purification and properties of a new β-lactamase from *Pseudomonas cepacia*. Antimicrob. Agents Chemother. **17:**355–358.
6. **Livermore, D. M., P. Y. Chau, A. I. Wong, and Y. K. Leung.** 1987. Beta-

lactamase of *Pseudomonas pseudomallei* and its contribution to antibiotic resistance. J. Antimicrob. Chemother. **20:**313–321.
7. **Massova, I., and S. Mobashery.** 1998. Kinship and diversification of bacterial penicillin-binding proteins and β-lactamases. Antimicrob. Agents Chemother. **42:**1–17.
8. **Nielsen, H., J. Engelbrecht, S. Brunak, and G. von Heijne.** 1997. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng. **10:**1–6.
9. **Scharf, S. J., G. T. Horn, and H. A. Erlich.** 1986. Direct cloning and sequence analysis of enzymatically amplified genomic sequences. Science **233:**1076–1078.
10. **Seoane, A., and J. M. Garcia-Lobo.** 1991. Nucleotide sequence of a new class A beta-lactamase gene from the chromosome of *Yersinia enterocolitica*: implications for the evolution of class A beta-lactamases. Mol. Gen. Genet. **228:**215–220.
11. **Siu, L. K., P. L. Ho, K. Y. Yuen, S. S. Y. Wong, and P. Y. Chau.** 1997. Transferable hyperproduction of TEM-1 β-lactamase in *Shigella flexneri* due to a point mutation in the Pribnow box. Antimicrob. Agents Chemother. **41:**468–470.
12. **Siu, L. K., J. Y. C. Lo, K. Y. Yuen, P. Y. Chau, M. H. Ng, and P. L. Ho.** 2000. β-Lactamases in *Shigella flexneri* isolates from Hong Kong and Shanghai and a novel OXA-1-like β-lactamase, OXA-30. Antimicrob. Agents Chemother. **44:**2034–2038.
13. **So, S. Y., P. Y. Chau, Y. K. Leung, and W. K. Lam.** 1984. First report of septicaemic melioidosis in Hong Kong. Trans. R. Soc. Trop. Med. Hyg. **78:**456–459.
14. **Trépanier, S., A. Prince, and A. Huletsky.** 1997. Characterization of the *penA* and *penR* genes of *Burkholderia cepacia* 249 which encode the chromosomal class A penicillinase and its LysR-type transcriptional regulator. Antimicrob. Agents Chemother. **41:**2399–2405.
15. **Tzouvelekis, L. S., E. Tzelepi, P. T. Tassios, and N. J. Legakis.** 2000. CTX-M-type beta-lactamases: an emerging group of extended-spectrum enzymes. Int. J. Antimicrob. Agents **14:**137–142.