ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1997, p. 1460–1464 Vol. 41, No. 7
0066-4804/97/$04.00+0

Sequence Analysis and Enzyme Kinetics of the L2 Serine β-Lactamase from *Stenotrophomonas maltophilia*

T. R. WALSH,[1]* A. P. MACGOWAN,[2] AND P. M. BENNETT[1]

Bristol Centre for Antimicrobial Research and Evaluation (BCARE), Department of Microbiology and Pathology, Medical School, University of Bristol, Bristol BS8 1TD,[1] and Southmead Health Services, NHS Trust, Bristol BS10 5NB,[2] United Kingdom

Received 15 October 1996/Returned for modification 27 January 1997/Accepted 1 May 1997

The L2 serine active-site β-lactamase from *Stenotrophomonas maltophilia* has been classified as a clavulanic acid-sensitive cephalosporinase. The gene encoding this enzyme from *S. maltophilia* 1275 IID has been cloned on a 3.3-kb fragment into pK18 under the control of a P*tac* promoter to generate recombinant plasmid pUB5840; when expressed in *Escherichia coli*, this gene confers resistance to cephalosporins and penicillins. Sequence analysis has revealed an open reading frame (ORF) of 909 bp with a GC content of 71.6%, comparable to that of the L1 metallo-β-lactamase gene (68.4%) from the same bacterium. The ORF encodes an unmodified protein of 303 amino acids with a predicted molecular mass of 31.5 kDa, accommodating a putative leader peptide of 27 amino acids. Comparison of the amino acid sequence with those of other β-lactamases showed it to be most closely related (54% identity) to the BLA-A β-lactamase from *Yersinia enterocolitica*. Sequence identity is most obvious near the STXK active-site motif and the SDN loop motif common to all serine active-site penicillinases. Sequences outside the conserved regions display low homology with comparable regions of other class A penicillinases. Kinetics of the enzyme from the cloned gene demonstrated an increase in activity with cefotaxime but markedly less activity with imipenem than previously reported. Hence, the *S. maltophilia* L2 β-lactamase is an inducible Ambler class A β-lactamase which would account for the sensitivity to clavulanic acid.

Stenotrophomonas (*Xanthomonas*) *maltophilia* has recently emerged as an important nosocomial pathogen in immunocompromised cancer patients and transplant recipients. *S. maltophilia* has been documented as a cause of bacteremia, infections of the respiratory and urinary tracts, meningitis, serious wound infections, mastoiditis, epididymitis, conjunctivitis, and endocarditis. Risk analysis has shown that mechanically ventilated intensive care unit patients receiving antibiotics, especially carbapenems, are at increased risk of bacterial colonization or infection with *S. maltophilia* (25).

S. maltophilia has been reported to be resistant to the newer antibiotics normally employed to treat gram-negative infections, such as extended-spectrum cephalosporins, carbapenems, and aminoglycosides. Reports indicate that some, if not most, isolates are resistant to all of the β-lactams normally employed to treat such infections, including broad-spectrum β-lactams, such as ceftazidime (10, 27). All strains also showed resistance to the carbapenems, such as imipenem and meropenem. Studies with β-lactam–inhibitor combinations have shown that many strains are resistant to combinations of piperacillin-tazobactam and cefsulodin-tazobactam, with ticarcillin-clavulanate showing the greatest activity (10, 27). The documented increase in the resistance of *S. maltophilia* clinical islates to β-lactams, which are normally employed to treat infections caused by these bacteria, is a major cause of concern.

S. maltophilia produces at least two chromosomally mediated β-lactamases, L1, a metallo-β-lactamase (28), and L2, a cephalosporinase sensitive to clavulanic acid, both of which are coordinately controlled. Coordinated expression has been shown by coinducibility using a single β-lactam as the inducer, the expression of both enzymes increasing by several hundredfold (21). However, *S. maltophilia* mutants displaying high-level β-lactam resistance have been isolated in which expression of only the L2 enzyme was markedly raised. Not surprisingly, this strain showed high-level resistance to the cephalosporins and penicillins and moderate resistance to the carbapenems (1). Inhibition studies with the L2 enzyme showed it to be very sensitive to clavulanic acid, more so than other serine β-lactamase inhibitors (13), and this, together with its hydrolytic profile, has placed the enzyme in functional classification group 2e (4). Recent reports suggest that *S. maltophilia* may produce up to four coordinately controlled β-lactamases (15, 16), although these results have yet to be corroborated.

Here we describe the sequence analysis of the *S. maltophilia* L2 β-lactamase and present the kinetics of the purified enzyme from the cloned gene.

MATERIALS AND METHODS

Cloning and expression of the L2 β-lactamase gene. The L2 β-lactamase gene was cloned from *S. maltophilia* 1275 IID chromosomal DNA on a 3.3-kb fragment into pRW33 to create the recombinant pSJ (5a). The fragment carrying the L2 β-lactamase gene was recloned, by using an *Eco*RI restriction-ligation strategy, into high-copy-number plasmid pK18 (18) for sequencing; the subsequent plasmid was designated pUB5811. *Escherichia coli* SNO3 (*ampC8*) (12), when transformed with pUB5811, demonstrated resistance to kanamycin (30 μg/ml) but, surprisingly, not ampicillin (30 μg/ml), indicating that expression of the L2 β-lactamase gene was dependent on the cloning, suggesting that the open reading frame (ORF) encoding the enzyme is positioned near the *Eco*RI restriction sites. To increase the level of expression of the L2 enzyme, pUB5811 was partially digested with *Eco*RI and ligated into *Eco*RI-cut pRU833 (26). The ligation mixture was used to transform *E. coli* DH5α (7), and recombinants were selected with kanamycin and streptomycin. The majority of the recombinants, when expressed in *E. coli* SNO3, demonstrated an increase in the MIC of ampicillin from 32 to 1,024 μg/ml on addition of isopropyl-β-D-thiogalactopyranoside (IPTG), indicating that the expression of the L2 β-lactamase gene is under the control of the P*tac* promoter. One construct was designated pUB5840.

Sequencing of plasmid DNA. DNA sequence determination was performed with a Du Pont Genesis 2000 automated sequencer. Sequences were determined on both strands by using a custom primer walking strategy. Compilation of resulting DNA sequences, database searches, and sequence alignments were

* Corresponding author.

performed by using the LASERGENE suite of programs (DNASTAR, West Ealing, London, United Kingdom).

Purification of the L2 β-lactamase. For enzyme production, an overnight 10-ml culture of *E. coli* (pUB5840) was used to inoculate a 2-liter fresh culture and the culture was grown at 37°C to the mid-log phase. IPTG (35 μg/ml) was then added to the culture, which was further shaken for 12 h. The cells were harvested (12,000 × *g*, 30 min, 4°C), and preparation of the periplasmic extract of the L2 enzyme was carried out as described by Lindstrom et al. (11). The periplasmic solution was dialyzed overnight against 30 mM Tris-HCl (pH 7.0) buffer, and the proteins were precipitated with 60% ammonium sulfate. After the periplasmic extract was prepared, the protein was estimated (by sodium dodecyl sulfate-polyacrylamide gel electrophoresis) to be 15% pure. After centrifugation (22,000 × *g*, 2 h, 4°C), the protein pellet was redissolved in 100 ml of Tris-HCl buffer (pH 7.0). The solution was loaded onto an equilibrated Sepharose-S column, which was then washed with 6 column volumes of Tris-HCl buffer (pH 7.0). Protein was eluted with a 0 to 0.8 M NaCl gradient. The fractions containing significant quantities of L2 were eluted with 0.35 M NaCl. These fractions were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the purity of L2 was estimated to be 65% (results not shown). The activity of the enzyme was checked at various stages of preparation by using nitrocefin hydrolysis as an indicator.

β-Lactamase assays. Hydrolysis of β-lactam antibiotics was examined by UV spectrophotometric assays (Pharmacia LKB Ultrospec II) in 1-cm light path cuvettes with readings recorded at 10-s intervals for 5 min at a wavelength of optimal absorbance for the β-lactam ring of each drug (cephalothin and cephaloridine, 265 nm; cefamandole and cefotaxime, 262 nm; cefoxitin, 260 nm; carbenicillin, 235 nm; cloxacillin, 260 nm; aztreonam, 254 nm; ampicillin and benzyl penicillin, 233 nm; imipenem, 299 nm). Antibiotic solutions were prepared in 10 mM phosphate buffer, pH 7.0. For substrate profiles, each antibiotic was assayed at a concentration of 100 μM, except benzyl penicillin, ampicillin, and carbenicillin, each of which was assayed at 500 μM.

Nucleotide sequence accession number. The nucleotide sequence reported here (see Fig. 1) has been deposited in the EMBL database under accession no. Y08562.

RESULTS

Nucleotide sequence analysis of the L2 β-lactamase gene. The ORF encoding the L2 β-lactamase extends for 909 nucleotides and encodes a preprotein of 303 amino acids (Fig. 1). Upstream of the L2 β-lactamase ORF lie the ribosome-binding site (RBS) and a putative −10 promoter sequence (TTC AAT) but no obvious −35 promoter sequence. At 41 nucleotides downstream of the termination codon are two inverted repeat sequences (CGCGCGCATCCTGCGCGCG) representing a possible terminator for the transcription of the L2 β-lactamase gene. Typical of β-lactamases, the primary translation product has a strong hydrophobic N terminus. The predicted secreted peptide is thought to be generated by cleavage between two alanine residues (amino acid positions 27 and 28). The mature protein has a predicted pI of 8.2, in close agreement with that reported by Saino et al. (22), and a molecular mass of 28.5 kDa. Similar to that of the L1 β-lactamase gene, the codon preference strongly favors cystine (C) and guanidine (G) over uridine (U) and adenine (A) in the third positions. In L2, the codon preferences were as follows: NNA, 4.2%; NNU, 11.7%; NNC, 47.7%; NNG, 36.5%. These preferences are reflected in the high GC content throughout the ORF (71.6%), similar to that of the ORF encoding the L1 metallo-β-lactamase (68.4%) (28). Both codon preferences and the high GC/AT ratio are similar to those for genes from *Pseudomonas* spp. rather than genes from members of the family *Enterobacteriaceae.*

Predicted protein sequence of the L2 β-lactamase. The predicted amino acid sequence of the L2 enzyme was compared to those of other β-lactamases. Enzymes with significant similarity scores were shown to be class A enzymes from gram-negative bacilli (Fig. 2). The highest identity score (54% using the Lipman-Pearson protein alignment) was with the BLA-A β-lactamase from *Yersinia enterocolitica*, followed by TOHO-1 from *E. coli* (51%), SL911 from *Klebsiella oxytoca* (50%), CdAmpC from *Citrobacter diversus* (50%), D488 from *K. oxytoca* (49%), and MEN-1 from *E. coli* (47%) (3, 6, 8, 9, 19, 23). When the protein sequences are aligned (Fig. 2), the L2 β-lactamase displays very similar regions of consensus with the other class A enzymes, including the consensus sequences STXK, SDN, and KTG and the highly conserved glutamic acid residue located 34 residues downstream from the SDN loop.

```
GCGCTGGTCGGCTCTGGGGAGGGCTTCAATAATCGGGCGGTCCCCAGGAGAACCCGC
                        -10                    RBS

1.  ATGCTCGCCCGTCGCCGATTCCTGCAGTTCAGTGGTGCCGCTGTTGCTTCCTCGCTTGCC
     M  L  A  R  R  R  F  L  Q  G  S  G  A  A  V  A  S  S  L  A

61. CTGCCGTTGCTGGCCCCTGCGGCGGGCAAGACCGCCGCCAGTGCACCGACCGATGCCGCC
     L  P  L  L  A  R  A  A  G  K  T  A  A  S  A  P  T  D  A  A

121.CTCACCGCCGCCACCGACTTCGCCGCATTGGAAAAGGCTGTGCGCGGCCGCTTCGGCGTG
     L  T  A  A  T  D  F  A  A  L  E  K  A  V  A  G  R  F  G  V

181.ACCCTGCTTGATACCGCCAGCGGCCGCCGCATCGGTCATCGCCAGGACGAGCGTTTCCCG
     T  L  L  D  T  A  S  G  R  R  I  G  H  R  Q  D  E  R  F  P

241.ATGTGCAGCACGTTCAAGAGCGTGCTGGCCGCCACCGTGCTCAGCCAGGCCGAGCGCCAG
     M  C  S  T  F  K  S  V  L  A  A  T  V  L  S  Q  A  E  R  R

301.CCCGCGTTGCTGGACACGCGCGTGCCGGTGCGCGATGCCGATCTTCTCTCGCACGCGCCG
     P  A  L  L  D  T  R  V  P  V  R  D  A  D  L  L  S  H  A  P

361.GTCACCCGCCGCCACGCCGGCAAGGACATGACCGTGCGCGATCTGTGCCGTGCCACGATC
     V  T  R  R  H  A  G  K  D  M  T  V  R  D  L  C  R  A  T  I

421.ATCACCAGCGACAATACCGCCGCCAACCTGCTGTTCGGCGTGGTCGGTGGCCCGCCCGCG
     I  T  S  D  N  T  A  A  N  L  L  F  G  V  V  G  G  P  P  A

481.GTGACTGCGTTCCTGCGCAGCATCGGTGATGCGGTGAGCCGCACTGATCGCCTGGAGCCG
     V  T  A  F  L  R  S  I  G  D  A  V  S  R  T  D  R  L  E  P

541.GAGCTGAACAGCTTTGCCAAGGGCGATCCGCGCGACACCACCACGCCGGCGGCGATGGCT
     E  L  N  S  A  L  P  G  D  P  R  D  T  T  T  P  A  A  M  A

601.GCGACCCTGCAGCGCGTGGTGCTGGGCGAGGTGCTGCAGCTGGCGTCGCGGCAGCAGCTG
     A  T  L  Q  R  V  V  L  G  E  V  L  Q  L  A  S  R  Q  Q  L

661.GCCGATTGGCTGATCGACAACGAAACCGGCGACGCCTGCCTGCGCGCGGGCCTGGGCAAA
     A  D  W  L  I  D  N  E  T  G  D  A  C  L  R  A  G  L  G  K

721.CTGTGGCGCGTCCGTGACAAGACCGGCAGCAACGGCGAAGACGCGCGCAACGACATCGCC
     L  W  R  V  R  D  K  T  G  S  N  G  E  D  A  R  N  D  I  A

781.GTGCTGTGGCCCGTGGCCGGCGGCGCACCGTGGGTGCTGACGGCCTATCTGCAGGCAGGT
     V  L  W  P  V  A  G  G  A  P  W  V  L  T  A  Y  L  Q  A  G

841.GCGATCAGCTACGAGCAACGCGCTACCGTGCTGGCACAGGTGGGGCGGATTGCCGACCGG
     A  I  S  Y  E  Q  R  A  T  V  L  A  Q  V  G  R  I  A  D  R

961.TTGATCGGATGAGGTAACCGGGGTCATTCAGAAATGGCTTGCCCGATCGGTTCGGCGCGC
     L  I  G  *                                              IR

GCATCCTGCGCGCG
      IR
```

FIG. 1. Nucleotide and predicted amino acid sequences of the L2 β-lactamase gene from *S. maltophilia*. The inverted repeat (IR) sequence, the possible terminator, is underlined. The −10 box and the RBS are also underlined. The stop codon is indicated by the asterisk.

Hydrolytic activities of the L2 β-lactamase. As seen in Table 1, the partially purified L2 enzyme displayed the greatest activity against cephaloridine (relative activity, 100%), followed by ampicillin (91%) and cefotaxime (46%), demonstrating the ability of the L2 β-lactamase to hydrolyze extended-spectrum cephalosporins. These activities, apart from those for cefotaxime and imipenem, are comparable to those previously published for the L2 β-lactamase (5). The activity of the enzyme preparation from expression of the cloned gene in *E. coli* against imipenem is significantly less than those of purified preparations obtained from cell extracts of induced *S. maltophilia* (5). Compared to the hydrolytic activities of other group 2e enzymes also prepared from *E. coli* expressing the cloned gene (Table 1), the L2 β-lactamase shows greater activities against a broad range of β-lactams, particularly cefotaxime. The L2 β-lactamase was tested for inhibition by sulbactam and clavulanic acid. On incubation with the inhibitor for 20 min at 30°C and using 120 μM cephaloridine as a reporter substrate, sulbactam had a 50% inhibitory concentration of 4.0 μM whereas clavulanic had a 50% inhibitory concentration of <0.1 μM.

DISCUSSION

The fragment containing the L2 β-lactamase gene, when subcloned (pUB5811) and expressed in *E. coli* SNO3, failed to

```
S. malt L2          MLARRRFLQGSGAAVASSLALPLLARAAGKTAASAPTDAALTAATDFAALEKAVAGRFGV   60
Y. ent BLA-A        MKHSSLRRSLLLAGITLPLVSFALPAWANALPASVDKQLAELERNANGRLGVAMINTGNG   60
K. oxy SL911        MIKSSWRKIAMLAAAVPLLLASGA LWASTDAIHQKLTDLEKRSGGRLGVALINTADNSQ   60
K. oxy D488         STDAIHQKLTDLEKRSGGRLGVALINTADNSQIL-------------------------   34
E.coli TOHO-1       MMTQSIRRSMLTVMATLPLLFSSATLHAQANSVQQQLEALEKSSGGRLGVALINTADNSQ   60
E.coli MEN-1        QTADVQQKLAELERQSGGRLGVALINTADNSQIL-------------------------   34
C. div CdAmpC       MFKKRGRQTVLIAAVLAFFTASSPLLARTQGEPTQVQQKLAALEKQSGGRLGVALINTAD   60
consensus           ----------------------------A--------------------------------

S. malt L2          TLLDTASGRRIGHRQDERFPMCSTFKSVLAATVLSQAERRPALLDTRVPVRDADLLSHAP   120
Y. ent BLA-A        TKIL--------YRAAQRFPFCSTFKFMLAAAVLDQSQSQPNLLNKHINYHESDLLSYAP   112
K. oxy SL911        IL----------YRGDERFAMCSTSKVMAAAAVLKQSESNKEVVNKRLEINAADLVVWSP   109
K. oxy D488         ------------YRGDERFAMCSTSKVMAAAAVLKQSESNKEVVNKRLEINAADLVVWSP    82
E.coli TOHO-1       IL----------YRADERFAMCSTSKVMAAAAVLKQSESNKHLLNQRVEIKKSDLVNYNP   110
E.coli MEN-1        ------------YRADERFAMCSTSKVMAVAAVLKKSESEPNLLNQRVEIKKSDLVNYNP    82
C. div CdAmpC       RSQIL-------YRGDERFAMCSTSKTMVAAAVLKQSETQHDILQQKMVIKKADLTNWNP   113
consensus           ------------YR-DERF-MCST-K-M-AAAVL-QSE---------------DL----P

S. malt L2          VTRRHAGKDMTVRDLCRATIITSDNTAANLLFGVVGGPPAVTAFLRSIGDAVSRTDRLEP   180
Y. ent BLA-A        ITRKNLAHGMTVSELCAATIQYSDNTAANLLIKELGGLAAVNQFARSIGDQMFRLDRWEP   172
K. oxy SL911        ITEKHLQSGMTLAELSAATLQYSDNTAMNLIIGYLGGPEKVTAFARSIGDATFRLDRTEP   169
K. oxy D488         ITEKHLQSGMTLAELSAATLQYSDNTAMNLIIGYLGGPEKVTAFARSIGDATFRLDRTEP   142
E.coli TOHO-1       IAEKHVNGTMTLAELGAAALQYSDNTAMNKLIAHLGGPDKVTAFARSLGDETFRLDRTEP   170
E.coli MEN-1        IAEKHVDGTMSLAELSAAALQYSDNVAMNKLISHVGGPASVTAFARQLGDETFRLDRTEP   142
C. div CdAmpC       VTEKYVDKEMTLAELSAATLQYSDNTAMNKLLEHLGGTSNVTAFARSIGDTTFRLDRKEP   173
consensus           ---KH----MTLAEL-AA-LQYSDNTAMN-LI---GGP--VTAFARS-GD-TFRLDR-EP

S. malt L2          ELNSALPGDPRDTTTPAAMAATLQRVVLGEVLQLASRQQLADWLIDNETGDACLRAGLGK   240
Y. ent BLA-A        DLNTARPNDPRDTTTPAAMAASMNKLVLGDALRPAQRSQLAVWLKGNTTGDATIRAGAPT   232
K. oxy SL911        TLNTAIPGDERDTSTPLAMAESLRKLTLGDALGEQQRAQLVTWLKGNTTGGQSIRAGLPE   229
K. oxy D488         TLNTAIPGDERDTSTPLAMAESLRKLTLGNALGEQQRAQLVTWLKGNTTGGQSIRVGLPE   202
E.coli TOHO-1       TLNTAIPGDPRDTTTPLAMAQTLKNLTLGKALAETQRAQLVTWLKGNTTGSASIRAGLPK   230
E.coli MEN-1        TLNTAIPGDPRDTTSPRAMAQTLRNLTLGKALGDSQRAQLVTWMKGNTTGAASIQAGLPA   202
C. div CdAmpC       ELNTAIPGDERDTTSPLAMAKSLHKLTLGDALAGAQRAQLVEWLKGNTTGGQSIRAGLPE   233
consensus           -LN-AIPGD-RDTTTP-AMA-----LTLG-AL---QRAQLV-WLKGNTTG---IRAGLP-

S. malt L2          LWRVRDKTGSNGEDARNDIAVLWPVAGGAPWVLTAYLQAGAISYEQRATVLAQVGRIADR   300
Y. ent BLA-A        DWIVGDKTGSGDYGTTNDIAVLWPTK-GAPIVLVVYFTQREKDAKPRRDVLASVTKIILS   291
K. oxy SL911        SWVVGDKTGAGDYGTTNDIAVIWPED-HAPLVLVTYFTQPQQDAKNRKEVLAAAAKIVTE   288
K. oxy D488         SWVVGDKTGAGDYGTTNDIAVIWPEN-HAPLVLVTYFTQPQQDAKNRKEVLAAAAKIVTE   261
E.coli TOHO-1       SWVVGDKTGSGDYGTTNDIAVIWPEN-HAPLVLVTYFTQPEQKAERRRDILAAAAKIVTH   289
E.coli MEN-1        SWVVGDKTGSGDYGTTNDIAVIWPKD-RAPLILVTYFTQPQPKAESRRDVLASAAKIVTN   261
C. div CdAmpC       GWVVGDKTGAGDYGTTNDIAVIWPED-RAPLILVTYFTQPQQDAKGRKDILAAAAKIVTE   292
consensus           -WVVGDKTG-GDYGTTNDIAVIWP----APLVLVTYFTQP---A--R---LA-AAKIVT-

S. malt L2          LIG                                                          303
Y. ent BLA-A        QIS                                                          294
K. oxy SL911        GL-                                                          290
K. oxy D488         GL-                                                          263
E.coli TOHO-1       GF-                                                          291
E.coli MEN-1        GL-                                                          263
C. div CdAmpC       GL-                                                          294
consensus           G
```

FIG. 2. Alignment of the amino acid sequence of L2 with those of penicillinases giving the highest identity scores. Amino acids were termed a consensus when identity was shared by five of the seven sequences. Boldface letters show where the consensus is shared by the L2 sequence. Sources of data shown: *S. maltophilia* L2, this study; *Y. enterocolitica* BLA-A, reference 23; *K. oxytoca* SL911, reference 6; *K. oxytoca* D488, reference 19; *E. coli* TOHO-1, reference 8; *E. coli* MEN-1, reference 3; *C. diversus* CdAmpC, reference 9.

confer resistance to ampicillin, suggesting that the gene is positioned close to one of the *Eco*RI sites used to clone the fragment. The nucleotide sequence showed that the start codon was within 83 nucleotides of the cloning site, consistent with the loss of the promoter. This is supported by the evidence of an RBS and a −10 promoter consensus but no −35 consensus, indicating that expression of the L2 β-lactamase gene in the original clone may be dependent on a hybrid promoter.

TABLE 1. Hydrolytic profiles of group 2e cephalosporinases inhibited by clavulanic acid

Enzyme	Strain	Relative rate of hydrolysis[a]									
		LOR	LOT	PEN	AMP	CAR	CLX	FOX	TAX	ATM	IMP
L2	*Stenotrophomonas maltophilia* 1275 IID[b]	100	23.6	29	91	7.5	<0.01	0.04	46	2.1	0.004
L2	*Stenotrophomonas maltophilia* 1275 IID[c]	100	7	32	26	3.0	4.0	0.001	20	12	25
CepA	*Bacillus fragilis* CS30[d]	100	NR	1.0	NR	NR	NR	NR	NR	NR	NR
CblA	*Bacteroides uniformis* WAL-7088[e]	100	NR	10	NR	NR	NR	NR	NR	NR	NR
CfxA	*Bacteroides vulgaris* CLA341[f]	100	68	11	7.2	NR	NR	<0.01	1.0	NR	NR
Form II	*Citrobacter diversus* ULA-27[g]	100	5.9	14	5.9	3.1	<0.01	NR	NR	NR	0.01
	Proteus vulgaris RO104[h]	100	120	3.3	3.4	NR	NR	NR	13	NR	NR
BlaI	*Yersinia enterocolitica* Y56[i]	100	250	38	32	12	NR	NR	NR	NR	NR

[a] Abbreviations: LOR, cephaloridine; LOT, cephalothin; PEN, penicillin; AMP, ampicillin; CAR, carbenicillin; CLX, cloxacillin; FOX, cefoxitin; TAX, cefotaxime; ATM, aztreonam; IMP, imipenem; NR, not reported.
[b] This study.
[c] Reference 5.
[d] Reference 20.
[e] Reference 24.
[f] Reference 14.
[g] Reference 2.
[h] Reference 17.
[i] Reference 23.

Typical of most *Stenotrophomonas* genes, the ORF encoding the L2 enzyme displays a high GC/AT ratio (71.6:18.4) with a significant preference for cytosine or guanidine in the third position of the codon.

Amino acid sequence analysis of L2 β-lactamase indicates it is a class A enzyme, despite consistently being referred to as a cephalosporinase (5, 22). This is perhaps not too surprising, as most of the other group 2e cephalosporinases sensitive to clavulanic acid are also class A enzymes. Like other members of its structural class, the L2 β-lactamase contains the conserved class A motifs STXK, SDN, and KTG and the highly conserved glutamic acid residue positioned 34 residues upstream from the SDN loop. When the predicted amino sequence of the L2 enzyme was compared to those of other proteins, the highest identity scores were those of β-lactamases classified in functional group 2be (4). These enzymes included TOHO-1 from *E. coli*, SL911 from *K. oxytoca*, CdAmpC from *C. diversus*, D488 from *K. oxytoca*, and MEN-1 from *E. coli* (3, 6, 8, 9, 19, 23). These enzymes have been classified in group 2be on the basis of their ability to hydrolyze cefotaxime, which is thought to be due to the threonine residue at position 165, which interacts with the oxyimino group of the β-lactam (19). At this position, the L2 β-lactamase contains a leucine residue.

The hydrolytic data of the partially purified L2 enzyme from the cloned gene product largely agree with those previously published (5), apart from those for cefotaxime and imipenem. The relative activity against imipenem was dramatically different from that previously published (25%), being 0.004% (relative to cephaloridine). Such an obvious difference may be due to the previous L2 β-lactamase preparation being contaminated with minor quantities of the L1 metallo-β-lactamase, demonstrating that published hydrolytic data for a separated β-lactamase(s) prepared from cell extracts from a single strain of a bacterium should be regarded cautiously.

Clavulanic acid was shown to be a more potent inhibitor of the L2 enzyme than sulbactam, confirming data previously reported (13). These data show that β-lactam–inhibitor combinations like ticarcillin-clavulanic acid are slightly more effective than pipercillin-tazobactam and cefsulodin-tazobactam against *S. maltophilia* isolates (10, 27). Presumably, total inhibition cannot be achieved due to the poor activity of the serine β-lactamase inhibitors toward the L1 metallo-β-lactamase with its ability to hydrolyze a broad spectrum of β-lactams.

ACKNOWLEDGMENTS

We are grateful to Stephen Cartwright for donating the pSJ construct.

This work was funded by the Wellcome Trust (grant 038025/2/93/2/1.5).

REFERENCES

1. **Akova, M., G. Bonifiglio, and D. M. Livermore.** 1991. Susceptibility to β-lactam antibiotics of mutant strains of *Xanthomonas maltophilia* with high- and low-level constitutive expression of L1 and L2 β-lactamases. J. Med. Microbiol. **35:**208–213.
2. **Amicosante, G., A. Oratore, N. Franceschini, M. Maccarrone, R. Storm, M. Galleni, and J.-M. Frere.** 1988. *Citrobacter diversus* ULA-27 β-lactamase. Improved purification and general properties. Biochem. J. **254:**885–890.
3. **Barthélémy, M., J. Péduzzi, H. Bernard, C. Tancrède, and R. Labia.** 1992. Close amino acid sequence relationship between the new plasmid-mediated extended spectrum β-lactamase MEN-1 and chromosomally encoded enzymes of *Klebsiella oxytoca*. Biochim. Biophys. Acta **1122:**15–22.
4. **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.
5. **Cartwright, S. J., and S. G. Waley.** 1984. Purification of β-lactamases by affinity chromatography on phenylboronic acid-agarose. Biochem. J. **221:** 505–512.

5a. **Cartwright, S. J.** Unpublished data.

6. **Fournier, B., P. H. Lagrange, and A. Philippon.** 1996. β-Lactamase gene promoters of 71 clinical strains of *Klebsiella oxytoca*. Antimicrob. Agents Chemother. **40:**460–463.
7. **Hanahan, D.** 1983. Studies on the transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **45:**37–67.
8. **Ishii, Y., A. Ohno, H. Taguchi, S. Imajo, M. Ishiguro, and H. Matsuzawa.** 1995. Cloning and sequence of the gene encoding a cefotaxime-hydrolyzing class A β-lactamase isolated from *Escherichia coli*. Antimicrob. Agents Chemother. **39:**2269–2275.
9. **Jones, M. E., M. B. Avison, E. Damdinsuren, A. P. MacGowan, and P. M. Bennett.** 1994. Heterogeneity at the β-lactamase structural gene *ampC* amongst *Citrobacter* spp. assessed by polymerase chain reaction, potential for typing at a molecular level. J. Med. Microbiol. **41:**209–214.
10. **Laing, F. P. Y., K. Ramotar, R. R. Read, N. Alfieri, A. Kureishi, E. A. Henderson, and T. J. Louie.** 1995. Molecular epidemiology of *Xanthomonas maltophilia* colonization and infection in the hospital environment. J. Clin. Microbiol. **33:**513–518.
11. **Lindstrom, B. E., H. G. Boeman, and B. B. Steele.** 1970. Resistance of *Escherichia coli* to penicillins. J. Bacteriol. **101:**218–231.
12. **Normark, S., and L. G. Burham.** 1977. Resistance in *Escherichia coli* to penicillins, fine-structure mapping and dominance of chromosomal β-lactamase mutations. J. Bacteriol. **132:**1–7.
13. **Padhananga, S. L., P. J. E. Rowling, I. N. Simpson, and D. J. Payne.** 1996. Sensitivity of L-2 type β-lactamases from *Stenotrophomonas maltophilia* to serine active site β-lactamases inhibitors. J. Antimicrob. Chemother. **37:**394–396.
14. **Parker, A. C., and C. J. Smith.** 1993. Genetic and biochemical analysis of a

novel Ambler class A β-lactamase responsible for cefoxitin resistance in *Bacteroides* species. Antimicrob. Agents Chemother. **37:**1028–1036.
15. **Paton, R., R. S. Miles, and S. G. Amyes.** 1994. Biochemical properties of inducible β-lactamases produced from *Xanthomonas maltophilia*. Antimicrob. Agents Chemother. **38:**2143–2149.
16. **Payne, D. J., R. Cramp, J. H. Bateson, J. Neale, and D. Knowles.** 1994. Rapid identification of metallo- and serine β-lactamases. Antimicrob. Agents Chemother. **38:**991–996.
17. **Peduzzi, J., A. Reynaud, P. Baron, M. Barthelemy, and R. Labia.** 1994. Chromosomally encoded cephalosporinase-hydrolysing β-lactamase of *Proteus vulgaris* RO104 belongs to Amblers' class A. Biochim. Biophys. Acta **1207:**31–39.
18. **Pridmore, R.** 1987. New and versatile cloning vectors with kanamycin resistance markers. Gene **56:**309–312.
19. **Reynaund, A., J. Péduzzi, M. Barthélémy, and R. Labia.** 1991. Cefotaxime-hydrolysing activity of the β-lactamase of *Klebsiella oxytoca* D488 could be related to the threonine residue at position 140. FEMS Microbiol. Lett. **81:**185–192.
20. **Rogers, M. B., A. C. Parker, and C. J. Smith.** 1993. Cloning and characterization of the endogenous cephalosporinase gene, *cepA*, from *Bacteroides fragilis* reveals a new subgroup of Ambler class A β-lactamases. Antimicrob. Agents Chemother. **37:**2391–2400.
21. **Roster, S., and H. Mett.** 1989. Physiological studies of the regulation of β-lactamase expression in *Pseudomonas maltophilia*. J. Bacteriol. **171:**483–487.
22. **Saino, Y., F. Kabayashi, M. Inoue, and S. Mitsuhashi.** 1984. Purification and properties of inducible cephalosporinase from *Pseudomonas maltophilia*. Antimicrob. Agents Chemother. **25:**362–365.
23. **Seoane, A., and M. Garcia Lobo.** 1991. Nucleotide sequence of a new class A β-lactamase gene from the chromosome of *Yersinia enterocolitica*: implications for the evolution of class A β-lactamases. Mol. Gen. Genet. **228:**215–220.
24. **Smith, C. J., T. K. Bennett, and A. C. Parker.** 1994. Molecular and genetic analysis of the *Bacteroides uniformis* cephalosporinase gene *cblA*, encoding the species-specific β-lactamase. Antimicrob. Agents Chemother. **38:**1711–1715.
25. **Spencer, R. C.** 1995. The emergence of epidemic multi-antibiotic-resistant *Stenotrophomonas maltophilia* and *Burkholderia cepacia*. J. Hosp. Infect. **30**(Suppl.)**:**453–464.
26. **Ubben, D., and R. Schmitt.** 1987. A transposable promoter and transposable promoter probes derived from Tn*1721*. Gene **53:**127–134.
27. **Vartivarian, S., E. Anaissie, G. Bodey, H. Sprigg, and K. Rolston.** 1994. A changing pattern of susceptibility of *Xanthomonas maltophilia* to antimicrobial agents: implications for therapy. Antimicrob. Agents Chemother. **38:** 624–627.
28. **Walsh, T. R., L. Hall, S. J. Assinder, W. W. Nichols, S. J. Cartwright, A. P. MacGowan, and P. M. Bennett.** 1994. Sequence analysis of the L1 metallo-β-lactamase from *Xanthomonas maltophilia*. Biochim. Biophys. Acta **1218:** 199–201.