Isolation and Characterization of a Penicillinase from *Pseudomonas cepacia* 249

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*Pseudomonas cepacia* has an inducible β-lactamase which is responsible for its novel ability to catabolize β-lactam compounds. The gene encoding this enzyme, penA, was cloned from a genomic library of *P. cepacia* 249 on the broad-host-range cosmid pLAFR. This separated the penA gene from the gene encoding a second β-lactamase in *P. cepacia* 249. Expression of penA was inducible in an *Escherichia coli* host strain by low levels of penicillin. The 33,500-molecular-weight enzyme had penicillinase activity not inhibited by clavulanic acid or sulbactam and was highly active against piperacillin and azlocillin. In comparison with other inducible β-lactamases produced by gram-negative organisms, the penA enzyme had many properties which were similar to those of the penicillinase produced by *Alcaligenes faecalis*. It was unlike the *ampC*-type cephalosporinase produced by *Pseudomonas aeruginosa*.

*Pseudomonas cepacia* has become an increasingly important pathogen in certain patient populations, particularly in immunocompromised hosts and children with cystic fibrosis (12). These infections are difficult to treat owing to the intrinsic resistance of this species to most antibiotics and the lack of clinical response to the few antimicrobial agents with in vitro activity against *P. cepacia* (11). Inducible β-lactamase activity, a general property of *P. cepacia*, has been associated with increased resistance to β-lactam antibiotics in clinical isolates of *P. cepacia* (7). However, the specific enzyme responsible for this resistance has not been identified. Beckman and Lessie (1) found that inducible penicillinase activity in *P. cepacia* was associated with the ability of this species to hydrolyze penicillin and to utilize β-lactam compounds as a source of carbon. This novel property has not been associated with the β-lactamases of other gram-negative bacteria.

In *P. cepacia* 249, two separate β-lactamases have been identified: a penicillinase responsible for approximately 80% of the total β-lactamase activity of the strain and a second enzyme with primarily cephalosporinase activities (13). It is not known if the cephalosporinase, like the penicillinase, is preserved throughout the species. The precise role of these enzymes in the in vivo development of resistance to β-lactam antibiotics is unclear. However, it has become quite apparent to clinicians that *P. cepacia* infections are much less responsive to β-lactam antibiotic therapy than *Pseudomonas aeruginosa* infections in the same patient population. Although *P. aeruginosa* produces a number of β-lactamases, differences in the properties of the β-lactamases produced by these two species may be in part responsible for their differing response to antibiotics.

In this study, we sought to characterize the inducible penicillinase found in *P. cepacia*. In addition to comparing its physical properties with those of other β-lactamases, we were interested in studying the regulation of penicillinase expression in this species. Using cloning techniques we isolated the β-lactamase from *P. cepacia* 249 which is associated with the ability of the strain to metabolize penicillin. This penicillinase was found to differ significantly from the *ampC*-type chromosomal β-lactamases associated with the enterobacteria and *P. aeruginosa* (16) in respect to physical properties, substrate profile, and induction kinetics. We found that this *P. cepacia* gene could be expressed and induced in an *Escherichia coli* host strain, which will facilitate analysis of its mode of regulation.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids.** The *E. coli* and *P. cepacia* strains and plasmids used in this study are listed in Table 1.

**Media and reagents.** M9CAA and LB media used throughout these studies have been described previously (8). Antibiotics used for selection in *E. coli* were as follows (micrograms per milliliter): chloramphenicol, 50; kanamycin, 50; penicillin, 150; tetracycline, 30, and for *Pseudomonas* species, 100. Kanamycin, penicillin, and tetracycline were purchased from commercial suppliers. Azlocillin, aztreonam, carbenicillin, cefotaxin, ceftazidime, cephaloridine, cephalothin, clavulanic acid, imipenem, piperacillin, sulbactam, and YTR830-H were gifts from their manufacturers.

**Cloning techniques.** Genomic DNA was isolated from *P. cepacia* by the method of Marmur (17). Plasmids were isolated from *P. cepacia* by the Birnboim-Doly technique (3) and from *E. coli* by the methods previously described (8). Restriction endonucleases, T4 DNA ligase, and λ packaging extracts were purchased from commercial suppliers and used as recommended. The cloning techniques used have been described previously (8).

**Cloning penA.** A genomic library of *P. cepacia* 249 was prepared by ligating 249 DNA cleaved with EcoRI to the broad-host-range cosmide vector pLAFR (9). After in vitro packaging into bacteriophage λ particles with a commercially supplied extract (Amersham Corp., Arlington Heights, Ill.) and transfection into *E. coli*, transfecants expressing the Tc' marker from pLAFR were selected. These clones were then mobilized as a group via triparental mating into the Pdx' Lys' strain 249-2 by using the helper plasmid pRK2013 (9) to provide transfer functions. *P. cepacia* transconjugants resistant to penicillin and able to use penicillin as a carbon source were selected. Plasmid pTGL70 was isolated from one of these clones and consisted of a 22-kilobase (kb) insert of 249 DNA in the *EcoRI* site of pLAFR. This

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plasmid was then mobilized into *E. coli* HB101 by selecting for Tc" and screening for β-lactamase production. The plasmid was also used to transform *E. coli* SNO3, a strain without background β-lactamase activity (14).

The structural gene for this penicillinase, which we designated penA, was localized on pTGL70. This was done by cloning the 22-kb EcoRI fragment into the EcoRI site of pACYC184 and subsequently deleting several HindIII fragments (Fig. 1). A 1.1-kb EcoRI-HindIII DNA fragment originally from *P. cepacia* 249 was then cloned into the vector pMK16 (26). This plasmid was designated pASP71.

The expression of the cloned penA gene in *Pseudomonas* species was tested by moving the 1.1-kb EcoRI-HindIII fragment from pASP71 to a vector which could replicate in *Pseudomonas* species. This plasmid, pASP8645 (Fig. 2), was constructed by the following steps. An *Hae*II fragment from pACYC184 carrying Cm" was cloned into a derivative of pNS2300 (22) in which a 1.8-kb PstI fragment had been deleted; the TEM β-lactamase from pNS2300 was deleted by cleavage with *Xmn*I and Scal followed by blunt-end ligation, and a 3-kb HindIII fragment including the 1.1-kb EcoRI-HindIII penA gene from pASP71 was inserted into the HindIII site of the polylinker. This plasmid, with the *Pseudomonas* IncP2 replicon and the RK2 oriT, was then mobilized by pRK2013 into strain 249-2.

**Molecular weight determination.** To determine the molecular weight of the penA β-lactamase, we used pASP71 to transform *E. coli* HS3149. Using the method of Sancar et al. (24), the molecular weights of the plasmid-encoded proteins were determined. In brief, this was done by irradiating the Rec" strain HS3149 containing the plasmid to destroy chromosomal DNA and incubating the cells overnight in cycloserine (100 μg/ml). The cells were washed several times, [35S]methionine was added, and the mixture was incubated in...
for 30 min. The cell proteins were solubilized and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After being stained with Coomassie blue, the gels were treated with EnHance (New England Nuclear Corp., Boston, Mass.) and fluorographed. The molecular weights of the proteins encoded by penA plasmid pASP71 could be determined by comparing the proteins encoded by the vector plasmid pMK16, pASP71, and a control plasmid pTJS53 which expressed large amounts of the TEM-1 β-lactamase and served as an internal control.

**Preparation of cell extracts.** Crude cell extracts containing the β-lactamase activity were prepared from the periplasmic contents of 50-ml cultures of bacteria by the method described by Witholt et al. (28). Cells were washed in 200 mM Tris hydrochloride (pH 8.0), suspended in 2 ml of sucrose-Tris hydrochloride, and treated with 5 mM EDTA and 10 mg of lysozyme per ml. The cells were dialyzed with 2 volumes of water and incubated for 30 min. The resulting spheroplasts were pelleted at 8,000 rpm for 20 min (Sorvall SS-34 rotor; DuPont Instruments). The supernatant representing the periplasmic contents was then dialyzed overnight in 0.05 M phosphate buffer and used as a source of β-lactamase.

**Isolation of β-lactamase activity by hydroxylapatite binding.** Using the method described by Beckman (W. Beckman, Ph.D. thesis, University of Massachusetts, Amherst, 1981), the total β-lactamase activity of strain 249 was separated into two fractions by their differential binding to hydroxylapatite. Cell extracts from 249 and 249-2(pASP8645) were dialyzed exhaustively against distilled water. A sample of the extract containing approximately 40 mg of protein was added to a hydroxylapatite column (Bio-Rad Laboratories, Richmond, Calif.) equilibrated with 0.01 M PO₄ buffer, and step gradients from 0.01 to 0.5 M PO₄ were applied. Fractions (1 ml) were collected and screened for β-lactamase activity with nitrocefin (20). Pooled fractions with β-lactamase activity from the same region of the gradient were concentrated, and their substrate profile was determined.

**β-Lactamase assays.** The cell extracts described above were used for all assays. These were concentrated as necessary with polyethylene glycol, dialyzed, and standardized by protein content measured by the method of Bradford (4). β-Lactamase activity was analyzed following published guidelines (5). Results are expressed as specific activities representing nanomoles of substrate hydrolyzed per minute per milligram of protein. A Gilford Response model continuously recording spectrophotometer temperature controlled at 35°C was used. Penicillinase activity measured by the starch-iodide technique (19) was assayed over 5 to 10 min. Reaction rates were shown to be linear for the dilutions of the enzyme preparation used. Penicillin G was used as a substrate in concentrations ranging from 3.1 to 300 μg/ml. Kinetic parameters were determined by assaying the activity for these substrate concentrations and applying the Line-weaver-Burk equation (5). All assays were run in duplicate. A substrate profile was determined by using 100 μM of each antibiotic. The hydrolysis of cephalexin compounds was followed at the appropriate wavelength for each compound, allowing the assays to continue for 30 min when necessary.

The effects of various inhibitors of β-lactamase activity were measured by incubating the enzyme preparation and several different concentrations of the inhibitor for 10 min at 35°C before assay. Penicillin G at concentrations of 100, 75, 60, 50, 25, and 12.5 μg/ml plus clavulanic acid at concentrations of 10, 6.6, 3.3, and 1.65 μg/ml were used to test inhibition of activity. Similarly, penicillin at 50 μg/ml and sulbactam at 100 and 10 μg/ml were assayed for inhibitory effects. The β-lactamase inhibitor YTR830-H at concentrations of 125, 100, 75, 50, 25, and 2 μg/ml was added to penicillin G at 50 μg/ml.

**Inducibility studies.** The induction kinetics of total β-lactamase activity from strain 249 were determined over 18 h by comparing the specific activities of enzyme produced from paired cultures, one grown with penicillin G (150 μg/ml) and one without inducer.

The inducibility of the cloned penA as expressed in E. coli or P. cepacia was determined. Stationary-phase cultures grown in the presence of inducer were diluted with fresh medium and inducer. After a 2- to 4-h incubation, cell extracts were prepared and assayed for β-lactamase activity. Control cultures were treated identically but not exposed to the inducing antibiotic. The gratuitous inducer 6-aminopenicillanic acid (6-APA) was used to induce E. coli SNO3 and P. cepacia 249-2, which were susceptible to very low levels of penicillin and cefoxitin.

### RESULTS

**β-Lactamase activity in strain 249.** The ability of either penicillin or cefoxitin to induce β-lactamase expression in strain 249 was demonstrated as shown in Table 2. Induction by penicillin G was efficient and appeared to depend on the continued presence of the inducer (Fig. 3). There was no demonstrable penicillin G activity in the culture supernatant after the 18-h incubation. Cell extracts of strain 249 contained two distinct peaks of β-lactamase activity separated by hydroxylapatite binding, one of which was eluted at low ionic strength (0.0125 M PO₄) and the other which was eluted at higher ionic strength (0.125 M PO₄). Substrate profiles of the β-lactamase in these fractions showed primarily penicillinase activity at low ionic strength with specific activities of 84 nmol/min for penicillin G and 2.4 nmol/min for cephalexin. The β-lactamase associated with the second peak eluted at high ionic strength had both penicillinase and cephalosporinase activity with specific activities of 140 nmol/min with penicillin G as a substrate and 80 nmol/min with cephaloridine.

#### Properties of cloned penA enzyme.

The β-lactamase activity expressed by the plasmid pTGL70 was analyzed by genetic and biochemical techniques. The Pdm− strain 249-2 was complemented by pTGL70, because 249-2(pTGL70) could both hydrolyze and metabolize penicillin. The multiple

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**TABLE 2. Induction of β-lactamase activity**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Inducer (μg/ml)</th>
<th>Sp act (nmol/min per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Penicillin G</td>
<td>Cephaloridine</td>
</tr>
<tr>
<td>249</td>
<td>None</td>
<td>15</td>
</tr>
<tr>
<td>249</td>
<td>Penicillin (150)</td>
<td>93</td>
</tr>
<tr>
<td>249</td>
<td>Penicillin (2,000)</td>
<td>1,623</td>
</tr>
<tr>
<td>249</td>
<td>Cefoxitin (100)</td>
<td>159</td>
</tr>
<tr>
<td>SNO3</td>
<td>None</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>SNO3</td>
<td>6-APA (10)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>SNO3(pTGL70)</td>
<td>None</td>
<td>2.0</td>
</tr>
<tr>
<td>SNO3(pTGL70)</td>
<td>Penicillin (50)</td>
<td>66.0</td>
</tr>
<tr>
<td>249-2</td>
<td>None</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>249-2</td>
<td>6-APA (10)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>249-2(pASP8645)</td>
<td>None</td>
<td>2.6</td>
</tr>
<tr>
<td>249-2(pASP8645)</td>
<td>Penicillin (50)</td>
<td>1.2</td>
</tr>
<tr>
<td>249-2(pASP8645)</td>
<td>Cefoxitin (10)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>249-2(pASP8645)</td>
<td>6-APA (10)</td>
<td>1.9</td>
</tr>
</tbody>
</table>

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auxotrophic mutations in 249-2 (15) were not fully complemented by pTGL70. The Bla⁻ phenotype of 249-2 was nonrevertible; thus, the β-lactamase activity of 249-2(pTGL70) was due to genes present on pTGL70. Strain 249-2(pTGL70) was not entirely stable, presumably owing to a large amount of plasmid DNA with homology to 249-2. The activity of the penA gene was then studied in a Rec⁻ E. coli background as expressed on pTGL70, in pASP71, or in a P. cepacia background as subcloned on pASP8645. The cloned penA gene expressed in pASP8645 did not complement the Pdm⁻ mutation of 249-2 because 249-2(pASP8645) was unable to utilize penicillin as a carbon source.

Molecular weight of penA β-lactamase. Using the maxicell technique, we showed that pASP71 expressed a single new polypeptide not produced by the cloning vector pMK16 (Fig. 4). This protein had a molecular weight of 33,500, which was consistent with the 1.1-kb size of the DNA fragment containing penA which had been cloned from pTGL70. Previous studies by Beckman and Lessie (W. Beckman and T. G. Lessie, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, K128, p. 148) using a sucrose gradient technique had associated the penicillinase activity of strain 249 with an enzyme with a molecular weight of approximately 34,000, and the cephalosporinase activity with an enzyme with a molecular weight of 45,000. In addition, the β-lactamase activity associated with 249-2(pASP8645) was eluted from a hydroxyapatite column at low ionic strength (0.0125 M PO₄) and has predominantly penicillinase activity, further confirming that the penicillinase of strain 249 as described by Lessie and Gaffney (15) and had been cloned.

Induction of penA expression. The expression of β-lactamase activity after induction with penicillin, cefoxitin, or 6-APA by the parent strain 249 and the cloned derivatives SNO3(pTGL70) and 249-2(pASP8645) were compared (Table 2). Although the parent strain 249 had β-lactamase activity inducible by either penicillin or cefoxitin, the β-lactamase expressed by the subcloned derivative pTGL70 was only inducible by penicillin. Expression of penA in SNO3(pTGL70), for which the MIC of cefoxitin is <0.1 µg/ml, was not inducible by subinhibitory concentrations of that antibiotic. In an E. coli HB101 background for which the MIC of cefoxitin is higher, the specific activity of HB101 (with penicillin G as a substrate) was 17 nmol/min, that of HB101(pTGL70) was 26 nmol/min, and that of HB101(pTGL70) induced with cefoxitin was 16 nmol/min. Penicillin G, but not cefoxitin, could induce penA activity as expressed by pTGL70.

All the smaller derivatives of pTGL70 shown in Fig. 1 had constitutive penA expression. Regulatory functions present in strain 249 and plasmid pTGL70 were apparently deleted in the construction of pASP8645. The production of the penA enzyme as subcloned in pASP71 or pASP8645 was no longer inducible in an E. coli host. Similarly, penA expression in trans in strain 249-2(pASP8645) was not inducible by either penicillin or cefoxitin. Thus, a penicillin-inducible β-lactamase, but not the cefoxitin-inducible enzyme, was expressed by pTGL70.

Substrate profile. The substrate profile obtained for SNO3(pTGL70) is shown in Table 3. The enzyme was

![Figure 3](http://aac.asm.org)  
**FIG. 3.** Inducible β-lactamase activity from P. cepacia 249 after the addition of penicillin G (150 µg/ml) at 30 min (○) as compared with a control (□) with penicillin G as a substrate.

![Figure 4](http://aac.asm.org)  
**FIG. 4.** Autoradiograph of ³⁵S-labeled proteins isolated from E. coli HS3149 containing the following plasmids: (A) pASP71 (vector plus the penA β-lactamase); (B) pMK16 (cloning vector); (C) pTJS55 expressing the TEM β-lactamase seen at approximately 30,000 as compared with the molecular weight standards (shown at the right, × 10⁵).

### Table 3. Substrate profile of β-lactamase from strain SNO3(pTGL70)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Relative rate of hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillins</td>
<td></td>
</tr>
<tr>
<td>Penicillin G</td>
<td>100</td>
</tr>
<tr>
<td>Azlocillin</td>
<td>141</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>127</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>83</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>54</td>
</tr>
<tr>
<td>Cephalosporins*</td>
<td></td>
</tr>
<tr>
<td>Cephaloridine</td>
<td>3.9</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

* These reactions were followed for 30 min to detect any significant enzymatic activity.

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predominantly a penicillinase. Using the Lineweaver-Burk equation for concentrations of penicillin G ranging from 3.1 to 300 μM, we determined the following kinetic parameters for the hydrolysis of penicillin G were: $K_{m} = 12.7$ μM; and $V_{max} = 48.5$ μM/min. The enzyme was active over a pH range of 6.0 to 9.0 with an optimum at pH 7.0. The penA hydrolysis of 100 μM penicillin G was inhibited less than 0.1% by pCMB (100 μM) or NaCl (1 M). Dicloxacillin (100 μM) inhibited activity by 14.1%, and zinc sulfate inhibited activity by 44%. Clavulanic acid over a wide range of concentrations of both the inhibitor and the penicillin G substrate had no appreciable effect on the activity of the enzyme. Similarly, neither sulbactam nor YTR830-H inhibited the penA enzyme appreciably.

**DISCUSSION**

The production of β-lactamase is a common property of gram-negative bacteria. Although a variety of different plasmid and chromosomal enzymes have been isolated and characterized (25), the penA enzyme is unique in its association with a metabolic pathway which allows P. cepacia to metabolize penicillin. From the original observations of Lessie and co-workers (1), it was clear that strain 249 contained two separate β-lactamases with different molecular weights and activities. We separated the penA enzyme from the cephalosporinase species and isolated the hydrolytic enzyme expressed in pASP8645 from the other functions present in pTGL70 which facilitate the metabolism of penicillin. This should allow identification of the elements required for penicillin metabolism in future complementation experiments.

The penA enzyme has been preserved in this species in isolates retrieved from the soil and plants as well as from hospitalized patients (1). In recent screening studies, we have found homology to a penA DNA probe in 25 of 25 clinical isolates of P. cepacia from geographically diverse locations (A. Prince, unpublished data). These strains were from sputum cultures of patients with cystic fibrosis and from blood cultures of patients with nosocomial infections. Inducible penA activity, perhaps in addition to other β-lactamases, should be expected in P. cepacia isolates.

The penA enzyme is clearly different from the β-lactamase from P. cepacia GN11164 described by Hirai et al. (13). The GN11164 enzyme with a molecular weight of 23,000 and predominantly cephalosporinase activity was characteristic of other P. cepacia isolates found in Japan, but seems unlike the enzymes which have been studied from P. cepacia strains in the United States (7). In the studies by Chiesa et al. (7), individual β-lactamases were not isolated from clinical isolates of P. cepacia and it appeared that many of these strains contained more than one type of β-lactamase. These enzymes are likely to be chromosomal, as plasmid-mediated β-lactamase production is relatively infrequent in this species (A. S. Prince and N. B. Scott, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 992, 1984).

Although the multiplicity of β-lactamases in P. cepacia complicates their analysis, further study of the mode of regulation of the isolated penA enzyme can be performed in a Bla− E. coli host. The cloned enzyme was expressed at a similar level in E. coli SNO3 and in P. cepacia 249-2 without induction. penA expression by SNO3(pTGL70) was increased 30-fold after induction, a level which should be adequate to define other elements present in pTGL70 responsible for penA regulation. Differences in the inducibility of penA as expressed in strain 249 and as cloned in pTGL70 may be due to problems in the expression of a Pseudomonas gene in an E. coli host or due to disruption of the wild-type regulatory functions in the cloning process.

The induction kinetics of the penA enzyme differed significantly from what has been described for other gram-negative organisms containing ampC β-lactamases (16). The pattern of penA induction was more characteristic of a metabolic gene than these antibiotic resistance genes. Expression of penA was readily induced by small amounts of substrate, and gene expression stopped when the inducer was consumed. This type of kinetics is quite different from that found in P. aeruginosa (18). The chromosomal β-lactamase of P. aeruginosa is inducible, but the kinetics of induction are not very efficient. The very high concentrations of inducer, 4,000 μg of penicillin G per ml, required to initiate induction approximate the MIC of penicillin for this organism. In addition, there is a considerable lag time between exposure to the inducer and enzyme production (18).

The penA gene of P. cepacia was efficiently induced by 150 μg of penicillin per ml and has been shown to be maximally inducible by 20 mg of penicillin per ml (1). These differences in β-lactamase induction kinetics may explain some of the discrepancies of the responses of P. aeruginosa and P. cepacia to the subinhibitory concentrations of β-lactam antibiotics which are often present in a clinical setting. This would be particularly true within the lungs of a cystic fibrosis patient colonized with large numbers of these organisms. Since the overall permeability of P. cepacia and P. aeruginosa to β-lactam compounds has been found to be very similar (21), the ability to rapidly induce β-lactamase expression may be an important selective advantage for P. cepacia.

The penA β-lactamase, by physical properties, activity, and induction kinetics, seems unrelated to the ampC-type enzymes of enterobacteria or P. aeruginosa (2). It is clearly different by these same parameters from either of the β-lactamases of *Pseudomonas maltophilia* (23). However, it does have many properties in common with the β-lactamase isolated from *Alcaligenes faecalis* (10). This β-lactamase is a penicillinase with a molecular weight of 29,000. The reported values of $K_{m} = 8.7$ μM and $V_{max} = 39.4$ μM/min for penicillin for the *Alcaligenes* enzyme are quite close to the parameters we obtained for the penA enzyme. The substrate profiles of the two enzymes are alike, with more activity against piperacillin: 128% for *A. faecalis* and 127% for *P. cepacia*, as compared with 100% for penicillin. Neither enzyme is inhibited by either clavulanic acid or sulbactam. Interestingly, by 55 RNA homology studies, *P. cepacia* is more closely related to *A. faecalis* than to other gram-negative organisms including *P. aeruginosa* (27).

Further analysis of the penA β-lactamase will be useful to determine the clinical significance of this enzyme and whether its induction results in the failure of antimicrobial therapy. The association of this β-lactamase with the general ability of *P. cepacia* to metabolize β-lactam compounds suggests that this enzyme has a more basic function in this species than simple antibiotic resistance. More effective antimicrobial agents for *P. cepacia* infections might include agents which inactivate penA or prevent its induction.

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LITERATURE CITED


