ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1989, p. 259–263
0066-4804/89/030259-05$02.00/0

Vol. 33, No. 3

MINIREVIEWS

Characterization of β-Lactamases

KAREN BUSH

The Squibb Institute for Medical Research, P.O. Box 4000, Princeton, New Jersey 08540

β-Lactamases (EC 3.5.2.6) are enzymes responsible for many failures of antimicrobial therapy because of the hydrolysis of β-lactam antibiotics to inert and ineffective agents. The first of these enzymes was recognized by Abraham and Chain in 1940 (1), before the widespread use of penicillin in the treatment of bacterial infections. Although the original β-lactamase described was primarily effective at hydrolyzing penicillins, many related enzymes with a wide range of substrate specificities are now recognized. Almost as soon as a new β-lactam antibiotic is introduced into clinical usage, some previously unrecognized β-lactamase with the capability of destroying this activity is identified.

In 1973, Richmond and Sykes (35) presented a thorough review of the β-lactamase literature, including a very useful classification scheme for β-lactamases from gram-negative bacteria as they were understood at the time. Sykes and Matthew expanded this compilation in 1976 (44), including isoelectric focusing data as a major criterion for identification of β-lactamases. However, since that time, many more β-lactamases have been identified. In addition, the distinguishing properties of these enzymes have been reevaluated to include substrate and inhibitor profiles with compounds, such as cefotaxime and clavulanic acid, that were not available during the original evaluations.

Several recent reviews of β-lactamases have included detailed descriptions of the chromosomal β-lactamases (38; C. C. Sanders, *in* L. E. Bryan, ed., *Microbial Drug Resistance*, in press) and the plasmid-mediated enzymes (6, 31), particularly with respect to their contributions to resistance. An excellent compilation of epidemiological data is included in the latter reviews. However, no complete tabulation of β-lactamase characteristics for both chromosomal and plasmid-mediated enzymes has recently been attempted.

In an effort to update the classification of β-lactamases, I recently proposed a modified Richmond-and-Sykes scheme based primarily on biochemical characteristics (11). Using substrate and inhibitor profiles in addition to physical data, a compilation of distinguishing β-lactamase data is given elsewhere (12, 13) for both chromosomal and plasmid-mediated enzymes. The criteria used for these evaluations, based upon the recommendations of Bush and Sykes (14), are explained in this minireview.

SELECTION OF REPRESENTATIVE ENZYMES

β-Lactamases included in the compilation (12, 13) must have been produced by bacteria. Although β-lactamase activity from yeasts (32), blue-green algae (25), and mammalian kidney (24) has been reported, these enzymes have not been evaluated under the same kinds of conditions as those for the bacterial β-lactamases and are not considered further.

For inclusion in the classification scheme (12, 13), the enzyme described must contain only a single β-lactamase activity. Although this is an essential criterion for defining physical and kinetic properties, this requirement is becoming increasingly difficult to meet. β-Lactamases are now appearing in organisms that harbor plasmids coding for one to three β-lactamases, in addition to a chromosomal enzyme (31, 34, 40, 42).

Although it has been suggested that all gram-negative bacteria code for a species-specific chromosomally mediated β-lactamase (44, 45), several different chromosomal enzymes from the same strain have recently been identified (4, 5, 36, 37). Each of these has been included in the tables in references 12 and 13. However, when very similar β-lactamases from different strains of the same organisms were characterized, the enzyme with the most complete information was selected as the species representative.

SUBSTRATE PROFILES

Substrate profiles indicating the range of β-lactam antibiotics hydrolyzed should provide some of the most definitive characteristics of β-lactamases. For many antibiotics, hydrolysis parameters can be used to explain the microbiological activity observed in organisms that produce given enzymes. Ideally, a complete set of V_{max} (maximal hydrolysis rates) and K_m (binding affinity) values should be available for every β-lactamase (14, 27). For practical purposes, only hydrolysis rates are usually presented in extended compilations. Recent studies with a limited number of cephalosporinases (18, 19) have provided complete kinetic data for a selected number of substrates. For other β-lactamases, the original literature must be consulted for additional critical parameters, such as K_m, amount of β-lactamase, and specific activity of the enzyme, e.g., micromoles of substrate hydrolyzed per minute per milligram of protein. The latter values depend upon factors such as method of preparation, purity of enzyme preparations, plasmid copy number for plasmid-mediated β-lactamases, and state of repression for chromosomal enzymes.

V_{max} is the preferred parameter for comparison in substrate profiles; many β-lactamase data are presented as relative rates of hydrolysis obtained at a single high substrate concentration. The assumption is thus made that a saturating concentration of substrate gives a rate approaching V_{max}. However, many β-lactamases exhibit substrate inhibition at high substrate levels. If assayed at these high substrate concentrations, maximal hydrolysis rates can be underestimated.

Reference β-lactam antibiotics have been selected for comparative substrate profiles based on the following considerations. The standard penicillins, benzylpenicillin, ampicillin, carbenicillin, and cloxacillin, were included because of their ability to discriminate among the various penicillinases and broad-spectrum β-lactamases. For these enzymes, benzylpenicillin has been used as the historical reference

β-lactam for hydrolysis comparisons. For cephalosporinases, cephaloridine has been chosen as the reference, with cephalothin added for comparison. Cephaloridine, a substrate that can be hydrolyzed to some extent by most β-lactamases, should be included in all profiles. Although nitrocefin is used as a reference substrate for many comparative studies, few kinetic data have been reported for this substrate. Because of the novel β-lactamases that are now being identified after clinical use of the extended-spectrum cephalosporins, cefotaxime and ceftazidime are included in the hydrolysis profile. Aztreonam as a representative of the monobactam family and imipenem as a typical carbapenem are included in the profile to allow for the determination of novel enzymes that are specifically active against these classes of antibiotics.

INHIBITION PROFILES

Inhibitory characteristics can be just as important as substrate profiles in distinguishing among β-lactamases. Differences among these enzymes with respect to inhibition by clavulanic acid and aztreonam have been shown to be significant: cephalosporinases are more strongly inhibited by aztreonam than by clavulanic acid, whereas broad-spectrum β-lactamases and penicillinases generally have weaker affinities for aztreonam than for clavulanic acid (11). Sulbactam should be included because of its increasing use as a β-lactamase inhibitor in clinical settings. Cloxacillin has long been recognized as a good inhibitor of the cephalosporinases and thus has also been included in many comparative determinations.

Many reports have described the action of mechanism-based inhibitors such as clavulanic acid and sulbactam, inactivators that may react in a time-dependent manner with certain penicillinases and broad-spectrum β-lactamases (10, 15, 23). It is inappropriate to list a simple K_i value for these inhibitors, because the mode of action may be quite complex, such that a K_i value does not represent a meaningful interaction between inhibitor and enzyme (15, 47). However, K_i values for clavulanic acid and sulbactam are scattered throughout the literature. More useful comparisons of relative inhibitory activities may be evaluated by using a simple I_{50} value, the concentration of inhibitor required to inhibit enzymatic activity by 50% under defined assay conditions (substrate used, substrate concentration, time for preincubation of enzyme and inhibitor).

For inhibitors such as aztreonam or cloxacillin that may act simply as poor substrates, inhibition data given as K_i values are generally preferred over I_{50} values. Inhibition data for *p*-chloromercuribenzoate and EDTA were often reported in the older literature as simple "sensitive" (+) or "resistant" (−) designations, based upon a single concentration of inhibitor. Sodium chloride inhibitory profiles are no longer determined for many of the newer enzymes, although inhibition by chloride is a distinguishing characteristic of the oxacillin-hydrolyzing β-lactamases. *p*-Chloromercuribenzoate inhibitory activity indicates the presence of a catalytically important cysteine residue; inhibition by EDTA may suggest that the enzyme requires a metal ion for activity.

Inhibition by boronic acids (16) or iodopenicillanate (17) has been used to predict the presence of active site serine residues in β-lactamases. Although the enzymes studied appear to conform to the predictions, there is a lack of data on a wide variety of these enzymes.

PHYSICAL CHARACTERISTICS

Historically, molecular weight determinations were often reported on the basis of gel exclusion chromatography. Some data have also been obtained by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, a more reliable determination of molecular weight for monomeric proteins. Recently, more enzymes have been purified extensively, such that amino acid compositions or, even better, amino acid sequences of the homogeneous enzyme have become available. Amino acid sequences are also being determined more frequently from nucleotide sequences of β-lactamase genes (20, 41, 43).

Comparisons between the sequence data and the gel filtration data have often concluded that gel filtration tended to underestimate the molecular weights of the β-lactamases. For example, SHV-1 β-lactamase had been estimated to have a molecular weight of 17,000 by gel filtration (30) but was shown to have a weight of 28,800 on the basis of amino acid sequence (7). The size of the PSE-2 enzyme was likewise increased to 27,500 (20) from 12,400 (29), again on the basis of the primary sequence of the β-lactamase.

Isoelectric points can also vary from one laboratory to another. In the compilations given elsewhere (12, 13), any significant discrepancies are indicated. Comparative values are best determined within a single laboratory, by using a variety of known β-lactamases as internal standards. It is advisable to determine pI values for similar enzymes by running the preparations in adjacent lanes on the gel. Another technique used to determine whether two proteins have the same isoelectric point is to mix the two and evaluate whether one band or two appear.

Separation of β-lactamases into molecular classes represents the obvious classification scheme. Four classes have been proposed to date based upon molecular structure: Ambler's class A serine penicillinases and class B metalloenzymes (2), Jaurin and Grundstrom's class C serine cephalosporinases (22), and the recently proposed class D oxacillin-hydrolyzing serine β-lactamases (20). As new amino acid structures or active site sequences become available, assignment to these classes will become routine. However, at this time most β-lactamases remain unclassified on a molecular level.

GENERAL CLASSIFICATION

In Table 1, a general classification scheme is outlined, with designated categories for the β-lactamases described. Subtitles for each grouping are included to suggest substrate specificity and susceptibility to inhibition by clavulanic acid. An attempt has been made to include those enzymes of known molecular structure within a single group, with the hope that future related enzymes will fit into these categories. A detailed tabulation of enzymes fitting into these categories is given elsewhere (12, 13).

Group 1 β-lactamases, subtitled CEP-N, are those enzymes that preferentially hydrolyze cephalosporins and are not inhibited by 10 μM clavulanic acid. Group 2 β-lactamases include a variety of enzymes that are all inhibited by clavulanic acid. Group 2a enzymes, subtitled PEN-Y, are the classical penicillinases. Group 2b enzymes are the traditional broad-spectrum β-lactamases, BDS-Y. Group 2b′, EBS-Y, includes many β-lactamases related to group 2b but with the ability to hydrolyze the "extended-broad-spectrum" β-lactam antibiotics, such as cefotaxime, ceftazidime, or aztreonam. Groups 2c, CAR-Y, and 2d, CLX-Y, include

TABLE 1. General classification scheme for bacterial β-lactamases

Group	Subtitle	Preferred substrates	Inhibited by:		Representative enzyme(s)
			CA[a]	EDTA	
1	CEP-N	Cephalosporins	No	No	Chromosomal enzymes from gram-negative bacteria
2a	PEN-Y	Penicillins	Yes	No	Gram-positive penicillinases
2b	BDS-Y	Cephalosporins, penicillins	Yes	No	TEM-1, TEM-2
2b'	EBS-Y	Cephalosporins, penicillins, cefotaxime	Yes	No	TEM-3, TEM-5
2c	CAR-Y	Penicillins, carbenicillin	Yes	No	PSE-1, PSE-3, PSE-4
2d	CLX-Y	Penicillins, cloxacillin	Yes[b]	No	OXA-1, PSE-2
2e	CEP-Y	Cephalosporins	Yes	No	*Proteus vulgaris*
3	MET-N	Variable	No	Yes	*Bacillus cereus* II, *Pseudomonas maltophilia* L1
4	PEN-N	Penicillins	No	?[c]	*Pseudomonas cepacia*

[a] 10 μM clavulanic acid.
[b] Inhibition by clavulanic acid may occur at higher concentrations for some members of the group.
[c] Variable.

those penicillinases that also hydrolyze carbenicillin or cloxacillin, respectively. Group 2e, CEP-Y, includes a unique group of cephalosporinases that are inhibited by clavulanic acid and resemble penicillinases in immunological properties. In Group 3, MET-N, are the β-lactamases that require a metal ion for enzymatic activity; all are apparently not inhibitable by clavulanic acid. Group 4, PEN-N, has been added to the original Bush classification scheme (11) and includes a variety of penicillinases that are not inhibited by clavulanic acid.

NOVEL β-LACTAMASES

In view of the many descriptions of new enzymes that have been recognized within the last few years, a myriad of novel β-lactamases may be expected in the future. Although isoenzymes were initially recognized among mammalian proteins containing multiple subunits (28), isoenzymic forms of almost any enzyme, including β-lactamases, can certainly be expected to be identified. In 1986, Schultz and Richards showed that single-amino-acid changes in the threonine adjacent to the active site serine in TEM-2 resulted in at least 15 active enzymes capable of conferring resistance to ampicillin (39). If one considers the number of amino acid changes possible in a β-lactamase, the number of distinct proteins is almost infinite. Because β-lactamases have been described as "floppy" (46), it is quite likely that minor amino acid changes make little difference in binding or hydrolysis of a number of β-lactam substrates.

It has been recognized for over a decade that the difference between TEM-1 and TEM-2 β-lactamases is one amino acid substitution (3, 43), resulting in a change in isoelectric point but no major change in kinetic properties. However, in the past year sequence data have been reported for two novel β-lactamases, CTX-1 (TEM-3) and SHV-2, each possessing major kinetic differences from the presumed parent enzymes. Only minor amino acid changes were observed (8, 41): TEM-2 to TEM-3, Glu 102 → Lys and Gly 236 (213 in mature enzyme) → Ser; SHV-1 to SHV-2, Gly 213 → Ser. Although none of these residues had been previously implicated in the active site, it is likely that the substitution of Ser 213 resulted in enzymes that are more capable of hydrolyzing oxime-substituted molecules, such as cefotaxime and ceftazidime.

This kind of mutational event has apparently become more common and will inevitably result in a proliferation of new β-lactamases with similar isoelectric points and molecular weights being identified. Before any β-lactamase can now be claimed to be novel, it is suggested that the following guidelines be followed.

The purported new enzyme should be compared directly with known β-lactamases of similar behavior, especially with respect to isoelectric focusing behavior, substrate hydrolysis for key antibiotics, and inhibition by clavulanic acid as a minimal set of standards. To make this internally consistent, the comparisons should all be performed in the same laboratory under identical conditions. Of course, this also implies that strains producing novel β-lactamases as described in the primary literature must be made available to other investigators for reference.

Further characterization may include testing for plasmid production and correlating activity with an identified plasmid. DNA hybridization is a sensitive technique that has been proposed as an alternative to isoelectric focusing for identifying β-lactamases (9, 26, 33). Unfortunately, stringency conditions for hybridization, size of the gene probe, and specificity of probe are variables that can affect the final results (9, 11, 26, 33). Also, DNA probes have the possibility of identifying unexpressed or nonfunctional β-lactamase sequences. Gene probes, however, can be quite useful in providing important information concerning families of β-lactamases or epidemiology of related enzymes.

All major β-lactamases will eventually be sequenced, thus allowing meaningful correlations between structure and function to be made. However, the biological activity associated with the enzyme should be emphasized as the most relevant attribute to a novel β-lactamase.

Nomenclature for novel enzymes has become a problem. Unless structural information can be determined for every new enzyme, names will be assigned according to individual preferences, rather than according to systematic procedures. Examples of this are the CTX-1 and CAZ-1 β-lactamases that have subsequently been designated TEM-3 and TEM-5 (41). It is quite likely that a number of other recently described β-lactamases are derived from a TEM-type enzyme, especially considering the ubiquity of TEM β-lactamases throughout gram-negative bacteria. In addition, a number of enzymes have now been identified as SHV-1 derivatives (7, 8, 21; A. Bure, P. Legrand, G. Arlet, V. Jarlier, G. Paul, and A. Philippon, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 868, 1988; L. Gutmann, F. W. Goldstein, B. Ferre, N. Rizk, M. H. Nicolas, J. F. Acar, and E. Collatz, 28th ICAAC, abstr. no. 869, 1988). However, unless molecular or genetic information concerning a novel enzyme is available, it is

premature to assign a new enzyme to a specific family of β-lactamases.

Although β-lactamases have been studied quite extensively over the past 40 years, the current status of these enzymes represents one of the most interesting stages of their development. One may expect to identify many new β-lactamases as the use of extended-spectrum β-lactam antibiotics increases throughout the world. Researchers must be willing to examine these novel enzymes carefully and must approach any classification and characterization with flexibility.

ACKNOWLEDGMENTS

I thank D. P. Bonner and R. K. Flamm for their helpful comments regarding the manuscript.

LITERATURE CITED

1. **Abraham, E. P., and E. Chain.** 1940. An enzyme from bacteria able to destroy penicillin. Nature (London) **146:**837.
2. **Ambler, R. P.** 1980. The structure of β-lactamases. Philos. Trans. R. Soc. London B Biol. Sci. **289:**321–331.
3. **Ambler, R. P., and G. K. Scott.** 1978. Partial amino acid sequence of penicillinase coded by *Escherichia coli* plasmid R6K. Proc. Natl. Acad. Sci. USA **75:**3732–3736.
4. **Amicosante, G., M. C. Marinucci, N. Franceschini, M. I. Tizzani, B. Oliva, and A. Oratore.** 1987. Fractionation and characterization of two β-lactamases in *Citrobacter diversus* ULA-27 strain by chromatofocusing. J. Chromatogr. **403:**366–372.
5. **Amicosante, G., A. Oratore, B. Joris, M. Galleni, J.-M. Frere, and J. Van Beeumen.** 1988. Chromosome-encoded β-lactamases of *Citrobacter diversus*. Interaction with β-iodopenicillanate and labelling of the active site. Biochem. J. **254:**891–893.
6. **Amyes, S.** 1987. Plasmid-mediated beta-lactamases: relative clinical importance, p. 31–49. *In* D. Livermore (ed.), Beta-lactamases: current perspectives. Theracom, The Hague, The Netherlands.
7. **Barthelemy, M., J. Peduzzi, and R. Labia.** 1988. Complete amino acid sequence of p453-plasmid-mediated PIT-2 β-lactamase (SHV-1). Biochem. J. **251:**73–79.
8. **Barthelemy, M., J. Peduzzi, H. B. Yaghlane, and R. Labia.** 1988. Single amino acid substitution between SHV-1 β-lactamase and cefotaxime-hydrolyzing SHV-2 enzyme. FEBS Lett. **231:**217–220.
9. **Boissinot, M., J. Mercier, and R. C. Levesque.** 1987. Development of natural and synthetic DNA probes for OXA-2 and TEM-1 β-lactamases. Antimicrob. Agents Chemother. **31:**728–734.
10. **Bush, K.** 1988. β-Lactamase inhibitors from laboratory to clinic. Clin. Microbiol. Rev. **1:**109–123.
11. **Bush, K.** 1988. Recent developments in β-lactamase research and their implications for the future. Rev. Infect. Dis. **10:**681–690.
12. **Bush, K.** 1989. Classification of β-lactamases: groups 1, 2a, 2b, and 2b′. Antimicrob. Agents Chemother. **33:**264–270.
13. **Bush, K.** 1989. Classification of β-lactamases: groups 2c, 2d, 2e, 3, and 4. Antimicrob. Agents Chemother. **33:**271–276.
14. **Bush, K., and R. B. Sykes.** 1986. Methodology for the study of β-lactamases. Antimicrob. Agents Chemother. **30:**6–10.
15. **Cartwright, S. J., and S. G. Waley.** 1983. β-Lactamase inhibitors. Med. Res. Rev. **3:**341–382.
16. **Cartwright, S. J., and S. G. Waley.** 1984. Purification of β-lactamases by affinity chromatography on phenylboronic acid-agarose. Biochem. J. **221:**505–512.
17. **De Meester, F., J.-M. Frere, S. G. Waley, S. J. Cartwright, R. Virden, and F. Lindberg.** 1986. 6-β-Iodopenicillanate as a probe for the classification of β-lactamases. Biochem. J. **239:**575–580.
18. **Galleni, M., G. Amicosante, and J.-M. Frere.** 1988. A survey of the kinetic parameters of class C β-lactamases. Cephalosporins and other β-lactam compounds. Biochem. J. **255:**123–129.
19. **Galleni, M., and J.-M. Frere.** 1988. A survey of the kinetic parameters of class C β-lactamases. Penicillins. Biochem. J. **255:**119–122.
20. **Huovinen, P., S. Huovinen, and G. A. Jacoby.** 1988. Sequence of PSE-2 β-lactamase. Antimicrob. Agents Chemother. **32:**134–136.
21. **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.
22. **Jaurin, B., and T. Grundstrom.** 1981. ampc cephalosporinase of *Escherichia coli* K-12 has a different evolutionary origin from that of β-lactamases of the penicillinase type. Proc. Natl. Acad. Sci. USA **78:**4897–4901.
23. **Knowles, J. R.** 1985. Penicillin resistance: the chemistry of β-lactamase inhibition. Acc. Chem. Res. **18:**97–104.
24. **Kropp, H., J. G. Sundelof, R. Hadju, and F. M. Kahan.** 1982. Metabolism of thienamycin and related carbapenem antibiotics by the renal dipeptidase, dehydropeptidase-I. Antimicrob. Agents Chemother. **22:**62–70.
25. **Kushner, D. J., and C. Breuil.** 1977. Penicillinase (β-lactamase) formation by blue-green algae. Arch. Microbiol. **112:**219–223.
26. **Levesque, R. C., A. A. Medeiros, and G. A. Jacoby.** 1987. Molecular cloning and DNA homology of plasmid-mediated β-lactamase genes. Mol. Gen. Genet. **206:**252–258.
27. **Livermore, D. M.** 1985. Do β-lactamases 'trap' cephalosporins? J. Antimicrob. Chemother. **15:**511–514.
28. **Mahler, H. R., and E. H. Cordes.** 1971. Biological chemistry, 2nd ed., p. 174. Harper & Row, Publishers, Inc., New York.
29. **Matthew, M.** 1978. Properties of the β-lactamase specified by the *Pseudomonas* plasmid R151. FEMS Microbiol. Lett. **4:**241–244.
30. **Matthew, M.** 1979. Plasmid mediated β-lactamases of gram-negative bacteria: properties and distribution. J. Antimicrob. Chemother. **5:**349–358.
31. **Medeiros, A. A., and G. A. Jacoby.** 1986. Beta-lactamase-mediated resistance, p. 49–84. *In* S. F. Queener, J. A. Webber, and S. W. Queener (ed.), Beta-lactam antibiotics for clinical use. Marcel Dekker, Inc., New York.
32. **Mehta, R. J., and C. H. Nash.** 1978. β-Lactamase activity in yeast. J. Antibiot. **31:**239–240.
33. **Ouellette, M., G. C. Paul, A. M. Philippon, and P. H. Roy.** 1988. Oligonucleotide probes (TEM-1, OXA-1) versus isoelectric focusing in β-lactamase characterization of 114 resistant strains. Antimicrob. Agents Chemother. **32:**397–399.
34. **Reid, A. J., I. N. Simpson, P. B. Harper, and S. G. B. Amyes.** 1987. Identification and characterization of a novel β-lactamase TLE-2, encoded by plasmid pUK702. FEMS Microbiol. Lett. **44:**125–128.
35. **Richmond, M. H., and R. B. Sykes.** 1973. The β-lactamases of gram-negative bacteria and their possible physiological role. Adv. Microb. Physiol. **9:**31–88.
36. **Saino, Y., M. Inoue, and S. Mitsuhashi.** 1984. Purification and properties of an inducible cephalosporinase from *Pseudomonas maltophilia* GN12873. Antimicrob. Agents Chemother. **25:**362–365.
37. **Saino, Y., F. Kobayashi, M. Inoue, and S. Mitsuhashi.** 1982. Purification and properties of inducible penicillin β-lactamase isolated from *Pseudomonas maltophilia*. Antimicrob. Agents Chemother. **22:**564–570.
38. **Sanders, C. C.** 1987. Chromosomal cephalosporinases responsible for multiple resistance to newer β-lactam antibiotics. Annu. Rev. Microbiol. **41:**573–593.
39. **Schultz, S. C., and J. H. Richards.** 1986. Site-saturation studies of β-lactamase: production and characterization of mutant β-lactamases with all possible amino acid substitutions at residue 71. Proc. Natl. Acad. Sci. USA **83:**1588–1592.
40. **Sirot, D., J. Sirot, R. Labia, A. Morand, P. Courvalin, A. Darfeuille-Michaud, R. Perroux, and R. Cluzel.** 1987. Transferable resistance to third-generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*: identification of CTX-1, a novel β-lactamase. J. Antimicrob. Chemother. **20:**323–334.
41. **Sougakoff, W., S. Goussard, G. Gerbaud, and P. Courvalin.** 1988. Plasmid-mediated resistance to third-generation cephalo-

sporins caused by point mutations in TEM-type penicillinase genes. Rev. Infect. Dis. **10**:879–884.
42. **Spencer, R. C., P. F. Wheat, T. G. Winstanley, D. M. Cox, and S. J. Plested.** 1987. Novel β-lactamase in a clinical isolate of *Klebsiella pneumoniae* conferring unusual resistance to β-lactam antibiotics. J. Antimicrob. Chemother. **20**:919–921.
43. **Sutcliffe, J. G.** 1978. Nucleotide sequence of the ampicillin resistance gene of *Escherichia coli* plasmid pBR322. Proc. Natl. Acad. Sci. USA **75**:3737–3741.
44. **Sykes, R. B., and M. Matthew.** 1976. The β-lactamases of Gram-negative bacteria and their role in resistance to β-lactam antibiotics. J. Antimicrob. Chemother. **2**:115–157.
45. **Takahashi, I., K. Tsukamoto, M. Harada, and T. Sawai.** 1983. Carbenicillin-hydrolyzing penicillinases of *Proteus mirabilis* and the PSE-type penicillinases of *Pseudomonas aeruginosa*. Microbiol. Immunol. **27**:995–1004.
46. **Thatcher, D. R.** 1975. Progress on penicillinase. Nature (London) **255**:526.
47. **Tipton, K. F.** 1973. Enzyme kinetics in relation to enzyme inhibitors. Biochem. Pharmacol. **22**:2933–2941.