CARBENICILLIN-HYDROLYZING β-LACTAMASE PRODUCED BY *CORYNEBACTERIUM PSEUDODIPHTHERITICUM*

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Resistance to penicillins and cephalosporins is mostly due to the formation of β-lactamases. The β-lactamases specified by R-plasmids are now classified into several types based on their enzymological properties[1]. Of these enzymes, the type IV (carbenicillin-hydrolyzing or PSE type) enzyme is less frequently seen in clinical isolates. Recently, we reported that carbenicillin-hydrolyzing enzymes have been found in various species of bacteria, *Echerichia coli*[2], *Proteus mirabilis*[3], *Enterobacter cloacae*[4], and *Pseudomonas aeruginosa*[5], and in many plasmids of various compatibility groups.

However, the β-lactamase from Gram-positive bacteria with the exception of *Bacillus licheniformis*[6], *Bacillus cereus*[7] and *Staphylococcus aureus*[8], have not been reported in any detail. Here we report the enzymological properties of a β-lactamase produced by *Corynebacterium pseudodiphtheriticum*.

Seven drug resistant strains of *C. pseudodiphtheriticum* were isolated from hog kidney. These strains did not ferment glucose, maltose, or sucrose. Urease, catalase and the reduction of tellurite by these strains was positive. The above key characteristics provided the basis for identification of these organisms as *C. pseudodiphtheriticum* (*C. hofmannii*)[9].

The drug resistance levels and β-lactamase activities of these organisms are shown in Table 1. *C. pseudodiphtheriticum* C56 had the highest enzyme activity and was used as a source for purification of the β-lactamase. The organism was grown overnight in Gifu-Anaerobic-Medium (GAM, Nissui Pharmaceutical Co., Ltd.) supplemented with 7% of horse serum at 37°C. The resultant cultures were diluted to 10-fold with same medium and then grown at 37°C for 5 hours with shaking. The cell were harvested by centrifugation and washed with 50 mM sodium phosphate buffer (pH 7.0). Crude enzyme was extracted from the cells by sonication, followed by centrifugation at $10,000 \times g$ for 40 minutes. Further purification of the enzyme was carried out on DEAE-Sephadex A-50, by column chromatography and Sephadex G-200 gel filtration.

The isoelectric point (pI) of the enzyme was 6.74, determined by isoelectrofocusing on an Ampholine (pH range: 3.5~9.0; LKB, Stockholm, Sweden) and the molecular weight was estimated to be $14,000 \pm 1,000$ by gel filtration (Sephadex G-200). One of the techniques that has recently introduced to try to classify the identification of bacterial β-lactamases has been isoelectric focusing[10,11]. The pI of the carbenicillin hydrolyzing enzymes has been previously described as following: type PSE 1 (pI 5.7), type PSE 2 (pI 6.1), type PSE 3 (pI 6.9), type PSE 4 (pI 5.3), *S. aureus* (pI 9.7) and *B.*

Table 1. Levels of drug resistance and β-lactamase activity of *Corynebacterium hofmannii*.

Strain	MIC (μg/ml)									β-Lactamase activity (units/mg protein)
	PCG	ABPC	CBPC	PIPC	CLX	CER	TC	CM	SM	
C55	400	800	1,600	3,200	50	0.8	1.56	3.13	400	1.43
C56	200	400	1,600	1,600	12.5	0.8	1.56	3.13	400	1.97
C60	400	800	1,600	3,200	50	0.8	1.56	3.13	400	0.95
C61	200	400	1,600	1,600	12.5	0.8	1.56	3.13	400	1.68
C85	6.25	3.13	25	12.5	0.8	0.4	1.56	3.13	400	0
C86	50	50	400	100	6.25	0.8	1.56	3.13	3.13	0
C87	12.5	25	100	50	3.13	0.4	1.56	3.13	1.56	0

Enzyme activity is expressed as units per miligram protein when benzylpenicillin is used as a substrate.
PCG Benzylpenicillin, ABPC ampicillin, CBPC carbenicillin, PIPC piperacillin, CLX cloxacillin, CER cephaloridine, TC tetracycline, CM chloramphenicol, SM streptomycin.

Table 2. Hydrolysis of various β-lactam antibiotics by β-lactamase from *Corynebacterium hofmannii* C56 strain.

Antibiotic	*Km* (μM)	Vmax (relative)[a]	*Ki* (μM)[b]
Benzylpenicillin	33.6	100	—[c]
Ampicillin	47.3	133	—
Carbenicillin	19.5	90	—
Piperacillin	25.5	44	—
Cloxacillin	73.9	9	25
Cephaloridine	252.9	3	—
Clavulanic acid	—	—	33
Sulbactam	—	—	40

[a] Relative rates of hydrolysis are expressed as the percentage of benzylpenicillin.
[b] *Ki* values were determined with benzylpenicillin (50 to 100 μM) as a substrate.
[c] Without preincubation.
—; Not determined.

cereus (pI 9.1). The substrate profiles are shown in Table 2. Benzylpenicillin, ampicillin, carbenicillin and piperacillin were susceptible to enzymatic hydolysis, but cloxacillin and cephaloridine were relatively resistant. This β-lactamase profile is typical of a penicillinase (PCase). We have recently classified plasmid mediated PCases into five types, *i.e.*, type I, type II, type III, type IV and type V on the basis of substrate profile, enzymological properties and immunological specificity[1]. The β-lactamase profile of *C. pseudodiphtheriticum* C56 is closely related to that of type IV PCase (carbenicillin hydrolyzing enzyme), and differs from the typical PCases produced by Gram-positive bacteria[2~5,10]. The β-lactamase produced by *C. pseudodiphtheriticum* C56 was competitively inhibited by cloxacillin (1 mM), and was noncompetitively inhibited by clavulanic acid (0.01 mM) and sulbactam (0.1 mM), but was not inhibited by *p*-chloromercuribenzoate. Specific antibody to type IV PCase completely inhibited the activity of the C56 enzyme.

A 3.6 megadalton plasmid was cured from *C. pseudodiphtheriticum* C56 strain by treatment with ethidium bromide, resulting in the simultaneous elimination of resistance to both penicillin and streptomycin. The elimination frequency was about 7%.

Carbenicillin-hydolyzing β-lactamases (type IV) were reported oliginally for Gram-negative bacteria[2~5,10]. This is the first report of such as enzyme being produced by a Gram-positive organism. Previously we have demostrated a plasmid governing the formation of PCase type IV in *P. mirabilis* and the gene governing the formation of the PCase was found to be located on a transposon[4]. Detailed studies on the β-lactamase produced by *C. pseudodiphtheriticum* will be described elsewhere.

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