CEPHABACIN $M_{1\sim6}$, NEW 7-METHOXYCEPHEM ANTIBIOTICS OF BACTERIAL ORIGIN

I. A PRODUCING ORGANISM, FERMENTATION, BIOLOGICAL ACTIVITIES, AND MODE OF ACTION

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New 7-methoxycephem antibiotics were found in culture filtrates of a bacterium isolated from a plant and named cephabacin $M_{1\sim6}$. They are the first members of 7-methoxycephem antibiotics of bacterial origin. The producing organism was taxonomically characterized and identified as *Xanthomonas lactamgena* YK-431; other strains of this species have recently been reported to produce cephabacin F and H group antibiotics. Cephabacin $M_{1\sim6}$ exhibited moderate antibacterial activity against Gram-negative and Gram-positive bacteria. Cephabacin $M_{1\sim6}$ were as stable as cephamycin C to cephalosporinases. They showed inhibitory activity against a cephalosporinase of *Proteus vulgaris* GN 4413. The mode of action of cephabacin M_1 was examined using *Escherichia coli* and *Bacillus subtilis* as test organisms; primary lethal targets of cephabacin M_1 are penicillin-binding protein (PBP) 1 in *E. coli* and PBP 4 in *B. subtilis*.

We have recently reported 15 new cephem antibiotics with oligopeptide side chains at the C-3 position produced by two species of Gram-negative bacteria, *Lysobacter lactamgenus* YK-90 and *Xanthomonas lactamgena* YK-278 and YK-280[1~3]. The antibiotics were named cephabacins after cephem antibiotics of bacterial origin. They are classified into two types, cephabacin F and H group antibiotics, according to the presence or absence of a 7-formylamino substituent, respectively. The former are highly resistant to hydrolysis by various types of β-lactamases[4]. In this paper and the accompanying paper[5], we describe a new group of cephabacins, cephabacin $M_{1\sim6}$ (Fig. 1), which are the first members of 7-methoxycephem antibiotics of bacterial origin.

In 1971, A-16886 B (CMC*) and A-16884 A (7-methoxy CPC), the first 7-methoxycephem antibiotics, were reported to be produced by procaryotes, *Streptomyces clavuligerus* and *S. lipmanii*, respectively[6]; this discovery opened up a new era of screening for β-lactam antibiotics which had been believed to be only fungal metabolites until then. Since the discovery of these antibiotics, several other 7-methoxycephem antibiotics have been reported as metabolites of some *Streptomyces* species[7~9].

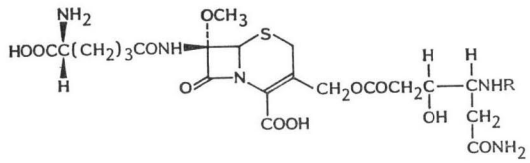
This paper deals with characterization of the producing organism, the fermentation, biological activities — such as antibacterial activity, stability to β-lactamases, and inhibitory activity against β-lactamases — and mode of action of cephabacin $M_{1\sim6}$.

Taxonomy of the Producing Organism

Strain YK-431 producing cephabacin $M_{1\sim6}$ was isolated on an agar plate containing colloidal

* Abbreviations of antibiotics used are: DACPC, deacetoxycephalosporin C; DCPC, deacetylcephalosporin C; CPC, cephalosporin C; CMC, cephamycin C.

Fig. 1. Structures of cephabacin $M_{1\sim6}$.



Cephabacin	R
M_1	←L-Val←L-Orn
M_2	←L-Val←L-Orn←L-Ser
M_3	←L-Val←L-Orn←L-Ser←L-Ala
M_4	←L-Val←L-Orn←L-Val←L-Orn
M_5	←L-Val←L-Orn←L-Val←L-Orn←L-Ser
M_6	←L-Val←L-Orn←L-Val←L-Orn←L-Ser←L-Ala

chitin as a sole carbon source from a plant sample obtained at Mine district in Yamaguchi Prefecture, Japan. Strain YK-431 is a Gram-negative, aerobic, lemon-yellow, polar-flagellated rod. It seems to have cellular pigment(s) giving a deep-blue color with concentrated sulfuric acid[10], but did not produce any diffusible pigment. The following characteristics of strain YK-431 were negative; reduction of nitrate, denitrification, methyl red test, Voges-Proskauer test, production of indole, H_2S and urease, and utilization of potassium nitrate. The following characteristics were positive; liquefaction of gelatin, hydrolysis of starch, utilization of citrate and ammonium sulfate, and production of oxidase and catalase. The oxidation-fermentation test of strain YK-431 was oxidative. The strain degraded colloidal chitin, carboxymethyl cellulose and Tween 80, but not alginate and agar. It utilized L-arabinose, D-xylose, D-glucose, D-mannose, D-fructose, D-galactose, maltose, sucrose, lactose, trehalose, D-sorbitol, glycerol, and starch as a sole carbon source, but did not utilize D-mannitol and inositol. It did not form acid or gas from any of these sugars. The guanine plus cytosine content (%) of DNA of strain YK-431 was 75.8±1.5 by the thermal denaturation method.

The above gross characteristics of strain YK-431 indicate that it is the same species as *X. lactamgena*, one of the producers of cephabacin F and H group antibiotics[1].

Fermentation

Seed culture for large-scale fermentation was performed by inoculating a loopful of cells into a 2-liter Sakaguchi flask containing 500 ml of a seed medium described before[1] and incubating the flask at 24°C for 48 hours on a reciprocal shaker (125 rpm). All of the seed culture was transferred into a 200-liter fermentor containing 120 liters of the seed medium supplemented with 0.05% of Actocol (an antiform, Takeda Chemical Industries, Ltd.). The seed culture was incubated at 24°C for 48 hours with an agitation rate of 150 rpm and an air flow of 120 liters/minute. Sixty liters of this culture was transferred into a 2,000-liter fermentor containing 1,200 liters of a large-scale fermentation medium consisting of dextrin 3%, soybean flour 1.5%, corn-gluten meal 1.5%, Polypepton (Daigo Nutritive Co., Ltd.) 0.2%, $Na_2S_2O_3 \cdot 5H_2O$ 0.1%, and Actocol 0.05% (pH not adjusted). The fermentation was carried out at 20°C for 66 hours with an agitation rate of 100 rpm and an air flow of 1,200 liters/minute.

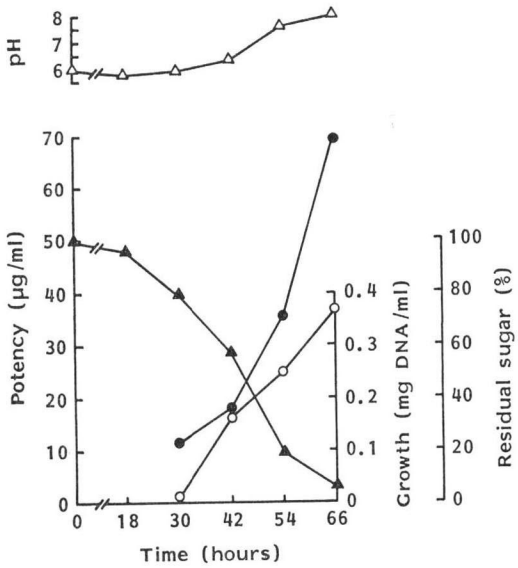
A typical fermentation profile is shown in Fig. 2. The total potency calculated as cephabacin M_1 was about 70 μg/ml. The major products of cephabacin M group antibiotics were components M_1

and M_4[5]. It is of interest that although strains YK-278 and YK-280 coproduce compounds with the 7-formylamino substituent, cephabacin F group antibiotics, and their corresponding non-substituted compounds, cephabacin H group antibiotics[1,2], strain YK-431 produces only compounds with the 7-methoxy substituent, cephabacin M group antibiotics.

DACPC and a compound sensitive to penicillinases, which was indistinguishable from penicillin N in mobilities on TLC-bioautography and HPLC, were detected only in cells of the early phase of the fermentation. These compounds disappeared in cells of the late phase accompanied by the accumulation of cephabacin M group antibiotics detected only in the culture filtrate (data not shown).

Fig. 2. Time-course of large-scale fermentation for cephabacin $M_{1\sim6}$ production.

Potency (●); calculated as cephabacin M_1 using *Pseudomonas aeruginosa* C141, a β-lactam hypersensitive mutant[1], as a test organism. Growth (○); the DNA content was determined by the method of BURTON[11] after extraction with 5% perchloric acid. Residual sugar (▲); determined by the glucose oxidase method. pH (△).



Antibacterial Activity

As shown in Table 1, cephabacin $M_{1\sim6}$ were moderately active against a wide range of Gram-negative and Gram-positive bacteria. Cephabacin M_1 having the shortest peptide side chain at the C-3 position showed the most potent activity. Because 7-methoxy DCPC showed far weaker antibacterial activity than cephabacin $M_{1\sim6}$ (Table 1), it is clear that the oligopeptide side chains of cephabacin M group antibiotics potentiate their antibacterial activity. The antibacterial spectra of cephabacin $M_{1\sim6}$ were intermediate between those of cephabacin F and H group antibiotics[4].

Stability to β-Lactamases

Cephabacin $M_{1\sim6}$ were far more stable against various types of cephalosporinases than was CPC (Table 2). As shown in Table 3, the stability of cephabacin M_1 to the enzymes was comparable to that of CMC and higher than that of cephabacin H_1 but lower than that of cephabacin F_1 with the 7-formylamino substituent. On the other hand, cephabacin H_1 was more stable to the enzymes than CPC. Hence, both the 7-methoxy substituent and the oligopeptide side chains at the C-3 position of cephabacin M group antibiotics seem to contribute to the stability against the cephalosporinases.

β-Lactamse Inhibitory Activity

We have developed a convenient assay system, St/Si system, to determine inhibitory activity against a cephalosporinase of *Proteus vulgaris* GN 4413[11]. Cephabacin $M_{1\sim6}$ and CMC showed potent inhibitory activity in this system whereas 7-methoxy DCPC and DCPC did not (Table 4).

In a cell-free assay system described previously[13], cephabacin M group antibiotics showed an inhibitory activity intermediate to those of cephabacin F and H group antibiotics[4] (data not shown). Cephabacin $M_{1\sim6}$ did not inhibit the activities of penicillinases of *Staphylococcus aureus* 1840 and

Table 1. Antibacterial activity of cephabacin $M_{1\sim6}$ and 7-methoxy DCPC.

Organism	MIC (μg/ml) at 10^6 cfu/ml						
	M_1	M_2	M_3	M_4	M_5	M_6	7-Methoxy DCPC
Escherichia coli NIHJ JC2	12.5	50	100	100	>100	>100	>100
Salmonella typhimurium IFO 12529	6.25	12.5	12.5	12.5	12.5	25	100
Citrobacter freundii IFO 12681	>100	>100	>100	>100	>100	>100	>100
Klebsiella pneumoniae IFO 3317	12.5	12.5	12.5	12.5	12.5	12.5	>100
Enterobacter cloacae IFO 12937	>100	>100	>100	>100	>100	>100	>100
Serratia marcescens IFO 12648	>100	>100	>100	>100	>100	>100	>100
Proteus mirabilis ATCC 21100	6.25	6.25	6.25	25	25	50	25
P. vulgaris IFO 3988	12.5	25	25	100	100	>100	50
P. morganii IFO 3168	12.5	25	12.5	25	25	25	>100
Pseudomonas aeruginosa IFO 3080	>100	>100	>100	>100	>100	>100	>100
Alcaligenes faecalis IFO 13111	3.13	6.25	3.13	12.5	12.5	12.5	12.5
Acinetobacter calcoaceticus IFO 12552	50	100	100	50	100	100	>100
Staphylococcus aureus FDA 209P	50	50	50	25	25	25	>100
Bacillus subtilis PCI 219	3.13	6.25	6.25	3.13	3.13	3.13	>100
B. megaterium IFO 12108	3.13	6.25	6.25	3.13	3.13	3.13	>100
Brevibacterium thiogenitalis ATCC 19240	6.25	12.5	12.5	6.25	6.25	6.25	>100

MICs were determined by 2-fold agar dilution method as described previously[12].

Table 2. Stability of cephabacin $M_{1\sim6}$ and CPC to cephalosporinases.

Source of enzyme	Unit/ml	Cephabacin						CPC
		M_1	M_2	M_3	M_4	M_5	M_6	
Enterobacter cloacae IFO 12937	1×10^{-3}	27	20.5	18.5	17	16	15	11
Proteus vulgaris GN 4413	1×10^{-2}	30.5	25	21	20	17	17	—
Escherichia coli 205 TEM R^+ (566)	1×10^{-3}	27	21.5	18.5	17.5	16	16	10
Pseudomonas aeruginosa U 31	1×10^{-3}	25.5	21	20.5	18	17	16	10.5
None		30	24.5	21	20	17	17	33

Stability to β-lactamases was assayed on nutrient agar plates seeded with *P. aeruginosa* C141, a β-lactam hypersensitive mutant[1], in the presence or absence of a cephalosporinase. The enzyme of *E. coli* 205 TEM R^+ (566) is a product of Boehringer Mannheim Co. (Germany) and the other enzymes were partially purified from cell lysates as described previously[13]. Figures in the table are diameters (mm) of inhibition zones. The concentrations of cephabacin $M_{1\sim6}$ and CPC tested were 100 and 10 μg/ml, respectively.

—; No inhibition zone.

Escherichia coli TN713 and a cephalosporinase of *Enterobacter cloacae* TN1282.

Mode of Action

The mode of action of cephabacins M_1 and M_4 was examined using *E. coli* LD-2 and *Bacillus*

Table 3. Comparison of stability of cephabacins M_1, F_1 and H_1, CMC, and CPC to cephalosporinases.

Source of enzyme	Unit/ml	Cephabacin			CMC	CPC
		M_1	F_1	H_1		
Enterobacter cloacae IFO 12937	1×10^{-2}	—	30.5	—	—	—
	1×10^{-3}	25	32	26.5	27	12.5
Proteus vulgaris GN 4413	1×10^{-2}	28	32	17	34.5	—
	1×10^{-3}	28	32	31	34	16.5
Escherichia coli 205 TEM R^+ (566)	1×10^{-2}	13	31	—	—	—
	1×10^{-3}	26	32	23.5	30	11
Pseudomonas aeruginosa U 31	1×10^{-2}	10	32	—	—	—
	1×10^{-3}	26	32	23.5	30	11.5
None		28	32	34	34.5	32

The experimental conditions, see the legend to Table 2. The concentrations of cephabacins, CMC, and CPC tested were 100, 100, and 10 μg/ml, respectively.

—; No inhibition zone.

Table 4. Inhibitory activity of cephabacin $M_{1\sim6}$ and related antibiotics against a cephalosporinase of *Proteus vulgaris* GN 4413.

Compound	Inhibition zone (φmm)	
	St	Si
Cephabacin M_1	—	21.5
M_2	—	20
M_3	—	20
M_4	—	21
M_5	—	21
M_6	—	20
CMC	—	32
7-Methoxy DCPC	—	—
DCPC	—	—

β-Lactamase inhibitory activity was assayed by comparing diameters of inhibition zones on plates (St) seeded with *Staphylococcus aureus* FDA 209P and plates (Si) seeded with the same organism, containing 0.002 unit/ml of a cephalosporinase of *P. vulgaris* GN 4413 and 2 μg/ml of cephaloridine[11]. The concentrations of the antibiotics tested were 100 μg/ml.

—; No inhibition zone.

Table 5. Antibacterial activity of cephabacins M_1 and M_4 and related antibiotics.

Compound	MIC (μg/ml) at 10^6 cfu/ml	
	Escherichia coli LD-2	*Bacillus subtilis* PCI 219
Cephabacin M_1	6.25	3.13
M_4	25	3.13
7-Methoxy DCPC	200	400
Cephabacin F_1	6.25	3.13
7-Formylamino DCPC	50	200
Cephabacin H_1	12.5	0.78
DCPC	25	3.13

MICs were determined by the 2-fold agar dilution method as described previously[12].

subtilis PCI 219 as test organisms. The antibacterial activity of the compounds used in this study is shown in Table 5.

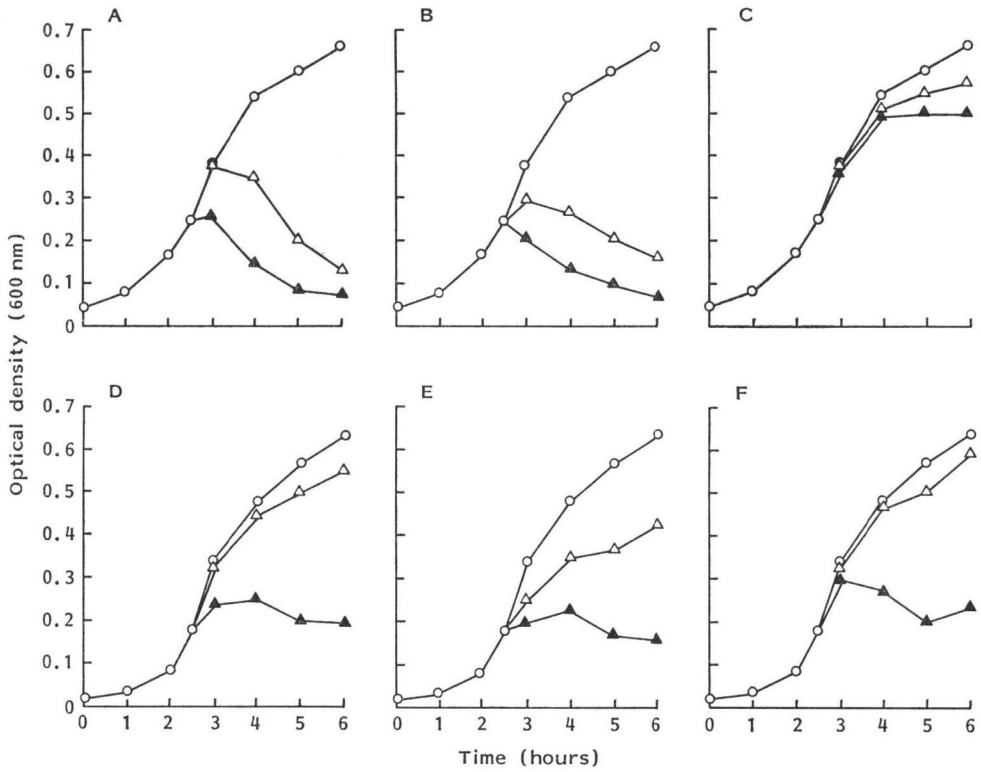
Cephabacins M_1 and M_4 showed strong lytic activity against *E. coli* LD-2 and *B. subtilis* PCI 219, whereas 7-methoxy DCPC had weaker activity (Fig. 3). In this experiment, morphological changes of *E. coli* LD-2 induced by cephabacins M_1 and M_4 were followed microscopically every hour. They formed spheroplasts leading to cell lysis, but never induced filamentous cells even under any concentrations tested (×0.1, ×1 and ×10 MIC).

The inhibitory activities of cephabacins M_1 and M_4 for peptidoglycan synthesis of *E. coli* LD-2 and *B. subtilis* PCI 219 were compared with those of several related antibiotics listed in Table 5. As shown in Fig. 4, cephabacin M_1 showed stronger inhibitory activity than cephabacin H_1 but weaker activity than cephabacin F_1 against *E. coli* LD-2. The inhibitory activity of cephabacin M_4 was similar to that of cephabacin H_1. PGI_{50} values defined as concentrations required to inhibit peptidoglycan synthesis by 50% of cephabacins M_1, M_4, F_1 and H_1, and 7-methoxy DCPC, 7-formylamino DCPC,

Fig. 3. Lytic activity of cephabacins M_1 and M_4, and 7-methoxy DCPC against *Escherichia coli* LD-2 and *Bacillus subtilis* PCI 219.

Cultures of *E. coli* LD-2 and *B. subtilis* PCI 219 grown in DYAB medium[12] to the exponential phase were diluted 5-fold with the fresh medium. Portions (4.5 ml) of the diluted cultures were delivered into sterilized tubes which were then incubated at 37°C with reciprocal shaking. After 2.5 hour-incubation, 0.5 ml of a solution of cephabacin M_1 (A, D), M_4 (B, E) or 7-methoxy DCPC (C, F) was added to the culture of *E. coli* LD-2 (A, B, C) or *B. subtilis* PCI 219 (D, E, F). Growth was followed by measuring the absorbance at 600 nm every hour with a Spectronic 20 colorimeter (Shimadzu, Bausch & Lomb).

○ Control, △ ×1 MIC of cephabacins M_1 and M_4 and 100 μg/ml of 7-methoxy DCPC, ▲ ×10 MIC of cephabacins M_1 and M_4 and 1,000 μg/ml of 7-methoxy DCPC.



and DCPC were 7.5, 23, 3.8, 19, 540, 98, and 54 μg/ml, respectively. Their minimum inhibitory concentrations (MICs) showed close agreement with their inhibitory activities for peptidoglycan synthesis in terms of PGI_{50} values, suggesting that the mode of action was similar in each (Fig. 6).

On the other hand, against *B. subtilis* PCI 219, cephabacin H_1 exerted the most potent inhibitory activity among the antibiotics tested. Cephabacins M_1, M_4, and F_1, and DCPC showed similar inhibitory activity, whereas 7-methoxy and 7-formylamino DCPC showed far weaker activity than the other cephem antibiotics (Fig. 5). The PGI_{50} values of cephabacin M_1, M_4, F_1, and H_1, 7-methoxy DCPC, 7-formylamino DCPC, and DCPC were 10, 5, 7.4, 1.5, 740, 245, and 9.6 μg/ml, respectively. There is also a parallel relationship between the MICs and the PGI_{50} values against this organism (Fig. 6).

The affinity of cephabacin M_1 for penicillin-binding proteins (PBPs) in *E. coli* LD-2 and *B. subtilis* PCI 219 was assayed by a competitive binding experiment with [^{14}C]benzylpenicillin as described

Fig. 4. Inhibition of peptidoglycan synthesis in *Escherichia coli* LD-2 by cephabacins M_1 and M_4, and related antibiotics.

Peptidoglycan synthesis was assayed by incorporation of [G-^{3}H]diaminopimelic acid (281 mCi/mmol, Amersham) into hot TCA-insoluble fraction as described previously[12].

○ Cephabacin M_1, ▼ cephabacin M_4, △ cephabacin F_1, □ cephabacin H_1, ● 7-methoxy DCPC, ▲ 7-formylamino DCPC, ■ DCPC.

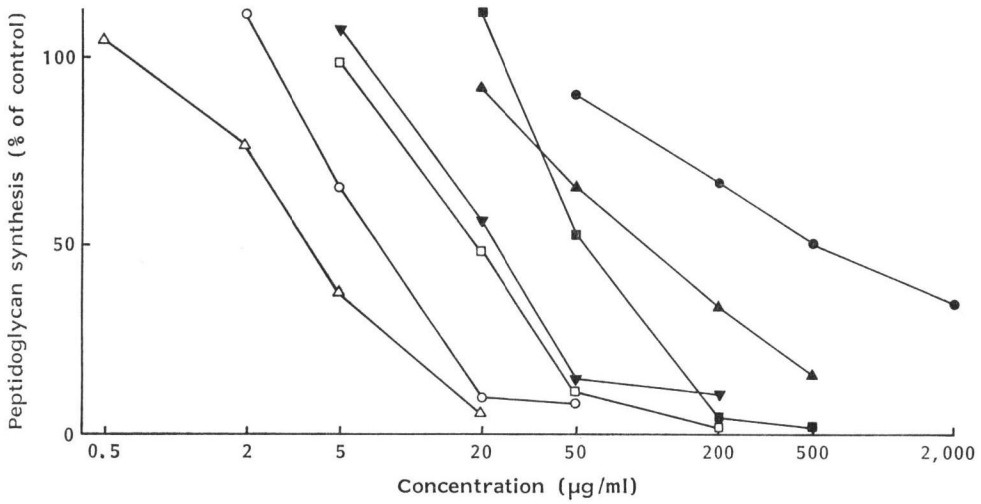
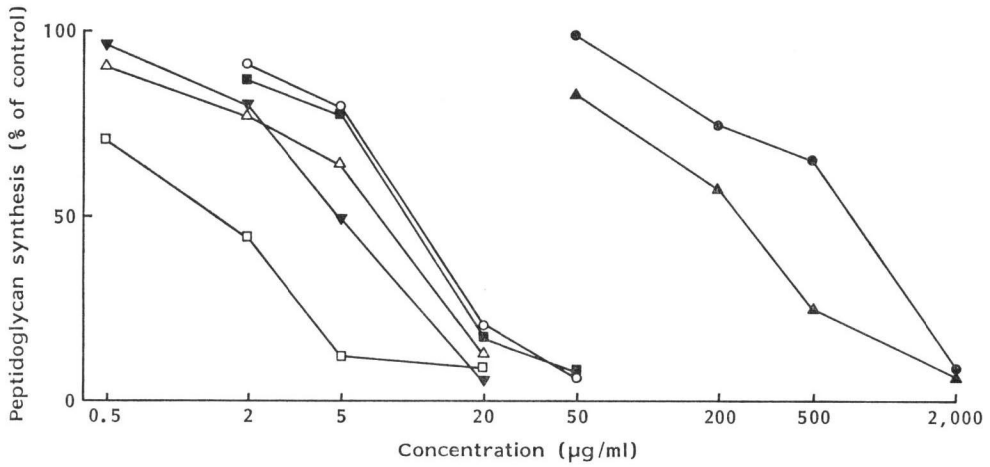


Fig. 5. Inhibition of peptidoglycan synthesis in *Bacillus subtilis* PCI 219 by cephabacins M_1 and M_4, and related antibiotics.

The experimental conditions and symbols of the antibiotics, see the legend to Fig. 4.



previously[12]. Among the lethal targets in *E. coli*, PBP 1, 2 and 3, cephabacin M_1 preferentially bound to PBP 1 (1A and 1B) (Fig. 7 and Table 6). Fig. 8 shows the comparison of the affinities of cephabacins M_1 and M_4, and 7-methoxy DCPC for the PBPs in *E. coli* LD-2. Cephabacin M_4 had a similar affinity profile to that of cephabacin M_1. In contrast, 7-methoxy DCPC showed far lower affinity for the PBPs than did cephabacins M_1 and M_4, reflecting its weak antibacterial activity against this organism. From these results, it is concluded that cephabacin M_1 and possibly all other cephabacin M

Fig. 6. Correlation between MICs and PGI_{50} values against *Escherichia coli* LD-2 and *Bacillus subtilis* PCI 219.

This figure is based on the data shown in Table 5 and Figs. 4 and 5.

○ *E. coli* LD-2, ● *B. subtilis* PCI 219.

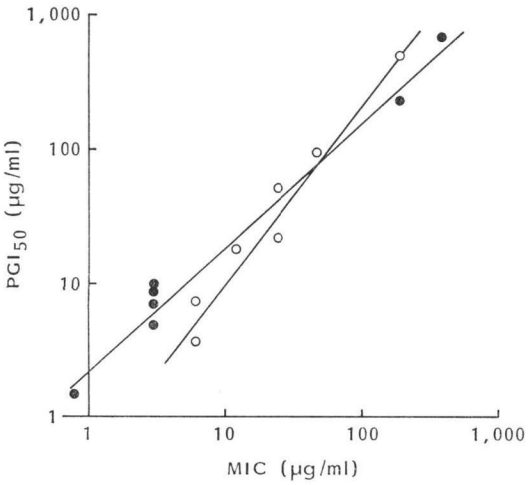
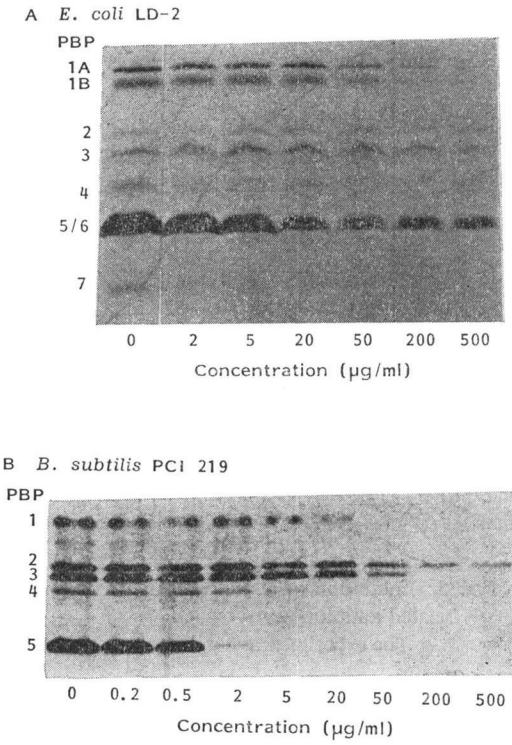


Fig. 7. Competition of cephabacin M_1 for [^{14}C]-benzylpenicillin binding to PBPs in *Escherichia coli* LD-2 and *Bacillus subtilis* PCI 219.

The assays of the affinity for PBPs in *E. coli* LD-2 and *B. subtilis* PCI 219 were carried out as described previously[12].



group antibiotics prevent the function of PBP 1 in *E. coli*, the main transpeptidase-transglycosylase involved in elongation of the murein sacculus, leading to the inhibition of peptidoglycan synthesis and to spheroplasting and cell lysis.

On the other hand, cephabacin M_1 showed the highest affinity for PBP 4 among the possible lethal targets, PBP 1, 2, and 4, in *B. subtilis* (Fig. 7 and Table 6)[14]. The I_{50} value of cephabacin M_1 for PBP 4 (2.3 μg/ml) was almost equal to that of cephabacin F_1 (1.8 μg/ml)[4]. These values were consistent with their MICs (3.13 μg/ml) against this organism (Table 5).

Cephabacin M_1 had high affinity for D-alanine carboxypeptidases, PBP 4~6 in *E. coli* and PBP 5 in *B. subtilis*[14]; this property appears to be common among 7-methoxycephem antibiotics[15~17].

Table 6. Affinities of cephabacin M_1 for each PBP in *Escherichia coli* LD-2 and *Bacillus subtilis* PCI 219.

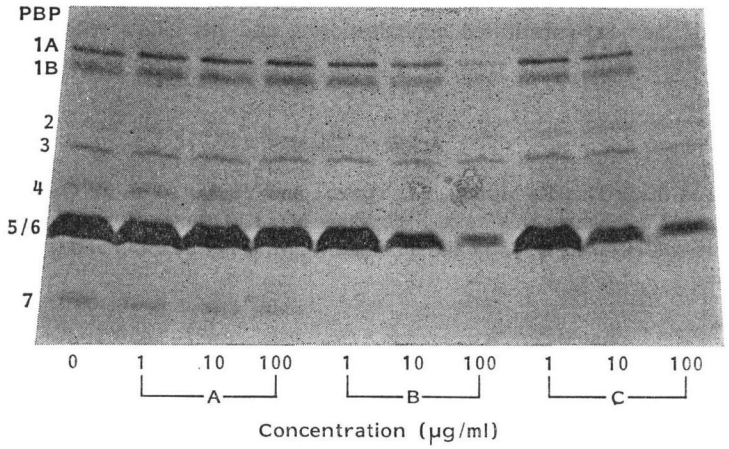
Organism	I_{50} (μg/ml)[a]								
	PBP								
	1A	1B	1	2	3	4	5	5/6	7
E. coli LD-2	37	30		>500	330	32		14	+[b]
B. subtilis PCI 219			3.7	32	29	2.3	1.0		

[a] I_{50}, a concentration required to inhibit binding of [^{14}C]benzylpenicillin to each PBP by 50%, was quantitated with the fluorograms shown in Fig. 7.

[b] The affinity for PBP 7 was positive but not quantitated.

Fig. 8. Comparison of affinities of cephabacins M_1 and M_4, and 7-methoxy DCPC for PBPs in *Escherichia coli* LD-2.

A, 7-Methoxy DCPC; B, cephabacin M_1; C, cephabacin M_4.



Discussion

In this report, we have described the first members of 7-methoxycephem antibiotics from bacteria. We have obtained 19 strains of *X. lactamgena* producing cephabacins. According to differences in taxonomical properties and the products of these strains, they have been classified into 3 types: 3 strains of the YK-278 type producing cephabacins $F_{4\sim9}$ and $H_{4\sim6}$; 15 strains of the YK-280 type producing cephabacins $F_{4\sim6}$ and $H_{4\sim6}$; and one strain of the YK-431 type producing cephabacin $M_{1\sim6}$. Therefore, the producers of cephabacin M group antibiotics seem to be rare in natural environments.

It should be noted that strain YK-431 does not produce a detectable amount of non-substituted compounds at the C-7 position corresponding to cephabacin $M_{1\sim6}$, whereas strains YK-278 and YK-280 types concomitantly produce cephabacin F and H group antibiotics[1~3], suggesting that the activities involved in the attachment of the 7-methoxy substituent may be high under our fermentation conditions. SINGH *et al.* reported the production of DACPC in cells of *Flavobacterium* sp.[18], a producer of 7-formylamino cephalosporins[19]. In contrast, we have previously reported that strains YK-278 and YK-280 produce DCPC in their cells[2]. According to our preliminary biosynthetic study, these strains also appear to have the ability to produce DACPC and penicillin N in cells depending on culture conditions or in the early phase of their fermentation as is the case with strain YK-431 described in this paper. Thus, the early biosynthetic pathway of cephabacin antibiotics is likely to be essentially the same as the biosynthetic pathway elucidated in fungi and *Streptomyces*[20]. In contrast, cephabacin antibiotics were exclusively detected in culture filtrates of the late phase of the fermentation. Hence, their fairly complex 3-side chains could be added to DCPC as they were transported from the cells to the culture medium.

Cephabacin M group antibiotics were stable against various types of β-lactamases to a degree comparable to that of CMC, but were more labile than cephabacin F group antibiotics, indicating that the 7-formylamino substituent more effectively confers cephem antibiotics stability against β-lactamases than does the 7-methoxy one. In general, both 7-formylamino and 7-methoxy substituents diminish antibacterial activity against Gram-positive bacteria. To date, several β-lactam antibiotics with these substituents have achieved a balance between antibacterial activity and β-lactamase stability and have been used therapeutically[21,22].

Our findings indicate that cephabacins M_1 and M_4 had the highest affinity for PBP 1 among the lethal targets in *E. coli*; this is in good agreement with their other biological activities. In contrast, 7-methoxy DCPC had a far lower affinity for the lethal targets in this organism, resulting in far weaker

antibacterial and biological activities against it. It seems very likely that the peptide side chains of cephabacin M group antibiotics potentiate their antibacterial activity due to an increased affinity for PBP 1. Cephabacins M_1 and M_4 showed a low affinity for PBP 2 and a high affinity for PBP 4~6 in *E. coli* LD-2. This affinity property is supposed to be due to the 7-methoxy substituent as elucidated by comparing with methoxy-substituted cephalosporins and penicillins with their non-substituted counterparts[15~17].

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References

1) ONO, H.; Y. NOZAKI, N. KATAYAMA & H. OKAZAKI: Cephabacins, new cephem antibiotics of bacterial origin. I. Discovery and taxonomy of the producing organisms and fermentation. J. Antibiotics 37: 1528~1535, 1984
2) HARADA, S.; S. TSUBOTANI, H. ONO & H. OKAZAKI: Cephabacins, new cephem antibiotics of bacterial origin. II. Isolation and characterization. J. Antibiotics 37: 1536~1545, 1984
3) TSUBOTANI, S.; T. HIDA, F. KASAHARA, Y. WADA & S. HARADA: Cephabacins, new cephem antibiotics of bacterial origin. III. Structural determination. J. Antibiotics 37: 1546~1554, 1984
4) NOZAKI, Y.; K. OKONOGI, N. KATAYAMA, H. ONO, S. HARADA, M. KONDO & H. OKAZAKI: Cephabacins, new cephem antibiotics of bacterial origin. IV. Antibacterial activities, stability to β-lactamases and mode of action. J. Antibiotics 37: 1555~1565, 1984
5) TSUBOTANI, S.; T. HIDA, H. ONO & S. HARADA: Cephabacin $M_{1\sim6}$, new 7-methoxycephem antibiotics of bacterial origin. II. Isolation, characterization and structural determination. J. Antibiotics 38: 1152~1165, 1985
6) NAGARAJAN, R.; L. D. BOECK, M. GORMAN, R. L. HAMILL, C. E. HIGGENS, M. M. HOEHN, W. M. STARK & J. G. WHITNEY: β-Lactam antibiotics from *Streptomyces*. J. Am. Chem. Soc. 93: 2308~2310, 1971
7) STAPLEY, E. O.; M. JACKSON, S. HERNANDEZ, S. B. ZIMMERMAN, S. A. CURRIE, S. MOCHALES, J. M. MATA, H. B. WOODRUFF & D. HENDLIN: Cephamycins, a new family of β-lactam antibiotics. I. Production by actinomycetes, including *Streptomyces lactamdurans* sp. n. Antimicrob. Agents Chemother. 2: 122~131, 1972
8) FUKASE, H.; T. HASEGAWA, K. HATANO, H. IWASAKI & M. YONEDA: C-2801X, a new cephamycin-type antibiotic. II. Isolation and characterization. J. Antibiotics 29: 113~120, 1976
9) INOUYE, S.; M. KOJIMA, T. SHOMURA, K. IWAMATSU, T. NIWA, Y. KONDO, T. NIIDA, Y. OGAWA & K. KUSAMA: Discovery, isolation and structure of novel cephamycins of *Streptomyces chartreusis*. J. Antibiotics 36: 115~124, 1983
10) SKERMAN, V. B. D.: A Guide to the Identification of the Genera of Bacteria. 2nd Ed., The Williams and Wilkins Co., Baltimore, 1967
11) BURTON, K.: Determination of DNA concentration with diphenylamine. Methods in Enzymol. 12B: 163~166, 1968
12) NOZAKI, Y.; A. IMADA & M. YONEDA: SCE-963, a new potent cephalosporin with high affinity for penicillin-binding proteins 1 and 3 of *Escherichia coli*. Antimicrob. Agents Chemother. 15: 20~27, 1979
13) OKONOGI, K.; Y. NOZAKI, A. IMADA & M. KUNO: C-19393 S_2 and H_2, new carbapenem antibiotics. IV. Inhibitory activity against β-lactamases. J. Antibiotics 34: 212~217, 1981
14) WAXMAN, D. J. & J. L. STROMINGER: Penicillin-binding proteins and the mechanism of action of β-lactam antibiotics. Ann. Rev. Biochem. 52: 825~869, 1983
15) OHYA, S.; M. YAMAZAKI, S. SUGAWARA, S. TAMAKI & M. MATSUHASHI: New cephamycin antibiotic, CS-1170: Binding affinity to penicillin-binding proteins and inhibition of peptidoglycan cross-linking reactions in *Escherichia coli*. Antimicrob. Agents Chemother. 14: 780~785, 1978
16) CURTIS, N. A. C.; G. W. ROSS & M. G. BOULTON: Effect of 7-α methoxy substitution of cephalosporins upon their affinity for the penicillin-binding proteins of *E. coli* K12. Comparison with antibacterial ac-

tivity and inhibition of membrane bound model transpeptidase activity. J. Antimicrob. Chemother. 5: 391~398, 1979

17) LABIA, R.; P. BARON, J. M. MASSON, G. HILL & M. COLE: Affinity of temocillin for *Escherichia coli* K-12 penicillin-binding proteins. Antimicrob. Agents Chemother. 26: 335~338, 1984

18) SINGH, P. D.; P. C. WARD, J. S. WELLS, C. M. RICCA, W. H. TREJO, P. A. PRINCIPE & R. B. SYKES: Bacterial production of deacetoxycephalosporin C. J. Antibiotics 35: 1397~1399, 1982

19) SINGH, P. D.; M. G. YOUNG, J. H. JOHNSON, C. M. CIMARUSTI & R. B. SYKES: Bacterial production of 7-formamidocephalosporins. Isolation and structure determination. J. Antibiotics 37: 773~780, 1984

20) DEMAIN, A. L. & N. A. SOLOMON: Antibiotics Containing the β-Lactam Structure. Springer-Verlag, Heidelberg, 1983

21) MITSUHASHI, S., *Ed.*: Beta-Lactam Antibiotics. Japan Sci. Soc. Press, Tokyo, Springer-Verlag, Berlin Heidelberg, New York, 1981

22) SLOCOMBE, B.; M. J. BASKER, P. H. BENTLEY, J. P. CLAYTON, M. COLE, K. R. COMBER, R. A. DIXON, R. A. EDMONDSON, D. JACKSON, D. J. MERRIKIN & R. SUTHERLAND: BRL 17421, a novel β-lactam antibiotic, highly resistant to β-lactamase, giving high and prolonged serum levels in humans. Antimicrob. Agents Chemother. 20: 38~46, 1981