

Aeromonas hydrophila によって産出されるオキサシリン型 ペニシリナーゼ

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Short Paper

Oxacillin-Hydrolyzing Penicillinase
Produced by *Aeromonas hydrophila*Fujio Hayashi*¹ and Matsuhisa Inoue*²

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Recently, we have extended our survey on drug-resistance of Gram-negative bacteria from the domestic animal and cultured fish. All of 150 *Aeromonas hydrophila* strains isolated from six kind of fresh-water fish in various districts of Gunma Prefecture were resistant to ampicillin (APC).¹⁾ We found two Inc C R-plasmids encoding APC resistance, Rms446 (TC, SA APC) and Rms447 (TC, SA, APC), from *A. hydrophila* strains in the intestinal tracts of eel *Anguilla japonica*. Rms446 and Rms447 plasmids were transferred to *Escherichia coli* K12 ML1410 but TC and APC resistance determinants on Rms447 were together lost in *A. hydrophila* HFM28 at a low frequency. Two plasmids were also transferred from *E. coli* K12 ML1410 to *A. hydrophila* HFM28 at a frequency of about 10^{-7} per donor strains in BHI broth.

Aoki and Egusa have discussed that APC resistance might be located on bacterial chromosome in this species.²⁾ Sawai *et al.* also reported that the APC resistance in *A. hydrophila* 67-P-24 and Y-62 could not transfer to *E. coli* K12 by conjugation, and APC resistance was not lost from the organisms after long storage of the strain in cooked-meat medium or by treatment with ethidium bromide. The β -lactamase (penicillinase, PCase: E.C.3.5.2.6) of *A. hydrophila* has a unique substrate specificity by hydrolyzing oxacillin, cephaloridine and ampicillin.³⁾ From these results, they have reported that *A. hydrophila* 67-P-24 and Y-62 produced the β -lactamase which is governed by APC resistance genes on chromosome.⁴⁾ However, APC resistance plasmids have not been reported until now. This paper is the first report on β -lactamase that are encoded by conjugative plasmids.

Crude β -lactamase extracts were prepared from *E. coli* K12 ML1410 and *A. hydrophila* HFM28 transconjugants carrying each R plasmid. Beta-lactamase activity was iodometrically determined at 20°C in 0.05 M phosphate buffer (pH 7.0) with 0.2 mM substrate by a modification of the Novick's method.⁵⁾ One unit of β -lactamase activity was defined as 1 μ M benzylpenicillin hydrolyzed in one min under the condition described above. The substrate specificity profile of β -lactamase mediated by these APC resistance plasmids is shown in Table 1. The β -lactamase mediated by plasmid Rms446 and Rms447 hydrolyzed carbenicillin and cloxacillin at a high rate. The enzymes

hydrolyzed ampicillin at a very high rate, about four times that of benzylpenicillin. There are four types of penicillin β -lactamase mediated by a plasmid. Plasmids Rms212, Rms213, Rtel6 and Rms139 encode type I (or TEM type),⁶⁾ type II,^{7,8)} type III (oxacillin hydrolyzing or OXA type),⁹⁾ and type IV (carbenicillin hydrolyzing or PSE type)^{10,11)} β -lactamases, respectively. The enzymes of Rms446 and Rms447 showed a quite similar substrate specificities to type II enzyme of plasmid Rms213.⁸⁾

APC resistance of *A. hydrophila* HF366 was not eliminated by treatment with ethidium bromide or after long storage of the strain, and the presence of plasmid was not confirmed in this strain. Accordingly, the existence of APC resistance genes on chromosome was estimated. It is assumed that β -lactamase of *A. hydrophila* HF366 is almost the same substrate specificity as reported by Sawai *et al.*³⁾ The substrate specificity profile of β -lactamase mediated by two R plasmids (Rms446 and Rms447) is similar to that of *A. hydrophila* chromosome.

Therefore, APC resistance determinants of two R plasmids may originate from chromosome genes of *A. hydrophila*.

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Table 1. Relative rate of hydrolysis of various penicillins by β -lactamase mediated by R-plasmids

Substrate	<i>A. hydrophila</i> HFM28* ¹		HF366* ²	<i>E. coli</i> ML1410* ¹		Rms213
	Rms446	Rms447		Rms446	Rms447	
Benzylpenicillin	100* ³	100	100	100	100	100
Ampicillin	400	370	341	409	536	450
Carbenicillin	99	70	95	90	63	80
Cloxacillin	111	76	83	79	51	292
Cephaloridine	30	48	40	33	41	263

*¹ Crude extract from cells of R⁺ strains; *A. hydrophila* HFM28 *met⁻pur⁻amp^s str.-r.*

*² *A. hydrophila* HF366 (wild type).

*³ Relative rate of hydrolysis (%).

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