AABOMYCIN A, A NEW ANTIBIOTIC. II

BIOLOGICAL STUDIES ON AABOMYCIN A

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The effects of aabomycin A on *Piricularia oryzae*, the causative agent of the rice blast disease and on the multiplication of Tobacco Mosaic Virus (TMV) were examined. Aabomycin A exhibited marked inhibition on the whole life cycle of *P. oryzae* including spore germination, mycelial growth and sporulation. The spore germination was the most influenced. The inhibitory activity of aabomycin A was not decreased in kasugamycin-resistant and blasticidin S-resistant clones. The rice blast disease on rice plants was remarkably suppressed by aabomycin A at a concentration of 20 ppm. It showed a protective effect rather than a curative effect, contrary to blasticidin S. No phytotoxicity was observed, even at a concentration of 1,000 ppm. The multiplication of TMV was remarkably inhibited by aabomycin A at a concentration of 1~100 mcg/ml and no phytotoxicity was observed even at a concentration of 1,000 mcg/ml.

It was reported in a previous paper[1] that aabomycin A is a water-insoluble, white needle crystal substance which inhibits the growth of *Piricularia oryzae* on agar plates at a concentration of less than 0.01 mcg/ml. There are quite a few examples of the water-insoluble antibiotics which have been used practically as agricultural fungicides. Aabomycin A is an excellent antibiotic, because of its high inhibitory activity on *Piric. oryzae* and low toxicity for fish and mammals. This paper reports on the inhibitory activity of aabomycin A on the rice blast disease as well as its effects on the multiplication of Tobacco Mosaic Virus (TMV).

Materials and Methods

Aabomycin A recrystallized from acetone-water was dissolved in ethanol or methanol followed by dilution with distilled water to certain concentrations before use.

Two-week old rice plant seedlings (Jukkoku) were used as test plants, and the inoculum of rice blast disease was harvested from the infected leaves of field-grown rice plants. The infected rice plant was treated with various concentrations of the antibiotic 2~4 days before inoculation to test the protective effect of the antibiotic. For curative effects, the antibiotic was sprayed 2 days after inoculation. The methods used were described by MISATO *et al.*[3]

The *in vitro* tests for the inhibitory effect on TMV multiplication was described

by HUANG *et al*[5,6], while quantitative analysis of TMV contents were according to the methods described by TANIGUCHI[4] and HUANG *et al.*[5]

Results

Antifungal activity of Aabomycin A on *Piricularia oryzae*

Piricularia oryzae strain P-2 was furnished by the Division of Plant Pathology, National Institute of Agricultural Science, Tokyo, for use as the test organism.

Effect of aabomycin A on the sporulation and germination of *P. oryzae* and on its mycelial growth were examined.

Table 1. Inhibition of sporulation, germination and vegetative growth of *Piricularia oryzae* with aabomycin A

Conc. of antibiotic (mcg/ml)	% Inhibition of		
	Sporulation[a]	Germination[b]	Vegetative growth[c]
Aabomycin A			
0.01	13	11	2
0.1	13	9	4
1.0	46	87	72
10.0	70	99	75
100.0	90	99	77
Blasticidin S			
0.01	30	3	49
0.1	39	10	50
1.0	51	15	60
10.0	68	94	81
100.0	86	100	95

a) Antibiotics were applied to a slant culture of *P. oryzae* 4 days after inoculation, and the number of spores was calculated 4 days after the antibiotic treatment.
b) Spore germination test was carried out according to the method described by MISATO *et al.*[2]
c) Antibiotics were added at the initial stage of log-phase (20 hours) and at the late stage of log-phase (50 hours). The mycelia were collected and their dry weights were measured.

Table 3. Effect of aabomycin A against rice blast (pot test *in vivo*)

Antibiotics	Concentration (ppm)	Preventive value (%)			
		A	B	C	D
Aabomycin A	20	48	67	88	55
	40	73	73	90	63
	80	72	81	95	62
	160	74	84	100	78
Blasticidin S	10	62	83	95	97
	20	78	78	97	99

A, B : pre-treatment of aabomycin A was carried out 4 and 2 days before inoculation in A and B respectively, to examine its protective effect.
C : direct effect.
D : post-treatment of aabomycin was carried out 2 days after inoculation to examine its curative effect.

Table 2. Effects of aabomycin A on the kasugamycin and blasticidin S resistant clones of *Piricularia oryzae*

(a)

Concentration (mcg/ml)	Diameter of inhibition zone in mm			
	Aabomycin A		Kasugamycin	
	S	Rk	S	Rk
0.1	9	9	—	—
1.0	14	14	—	—
10.0	26	29	9	9
100	32	37	12	9
1,000	—	—	15	9
10,000	—	—	30	9

(b)

Concentration (mcg/ml)	Diameter of inhibition zone in mm			
	Aabomycin A		Blasticidin S	
	S	Rb	S	Rb
0.1	9	13	—	—
1.0	16	18	9	9
10.0	25	30	17	9
100	33	35	35	15
1,000	—	—	50	30

Paper disc method was used. Medium contains yeast extract 0.5%, glucose 2%, pH 5 or 7 with (a) or (b) respectively.
S : sensitive clone of *P. oryzae*, P2 strain.
Rk : kasugamycin resistant clone of *P. oryzae*, P2 strain.
Rb : blasticidin S resistant clone of *P. oryzae*, P2 strain.

Table 4. Effect of rice plant juice on activity of aabomycin A against *Piricularia oryzae*

Concentration of aabomycin A (mcg/ml)	Diameter of inhibition zone (mm)		
	Control	Heated juice	Juice
0	0	0	0
0.5	10.0	10.5	10.5
5.0	19.5	21.0	21.3
50.0	27.0	31.0	30.7

Aabomycin containing 10% rice plant juice was incubated on tube shaker for 2 hours at 28℃, and inhibition zone was measured by paper disc method. Rice plant juice was prepared by homogenenizing 20 g of rice plant in 80 ml H_2O with Waring blenders (3,000 rpm, 2 minutes), filtering through cheese cloth, then centrifuging at 3,000 rpm for 15 minutes.

As shown in Table 1, aabomycin A exhibited inhibitory effects on each step of the growth cycle of *P. oryzae*, and the spore germination seemed to be the most inhibited stage. It was characteristic that the effect of aabomycin A decreased suddenly at concentrations below 0.1 mcg/ml.

Clones of *P. oryzae* resistant against kasugamycin and blasticidin S respectively were examined. Aabomycin A inhibited the growth of the resistant as well as the sensitive clones at the same concentration (Table 2).

Table 3 shows the protective and curative effect of aabomycin A against *P. oryzae* strains *in vivo* (pot test). Contrary to blasticidin S, aabomycin exhibited protective effect rather than curative effect. Considerably higher concentrations, more than 40 ppm, were required for complete inhibition in the pot test, though inhibitory effects *in vitro* were observed at the concentration as low as 0.1 mcg/ml.

These results suggested that some factors affected the stability of the antibiotic on the rice plants. Because aabomycin A is stable against ultraviolet irradiation and acid or alkali (pH 2~9) at room temperature, it might be considered that the breakdown, transformation or inactivation of the antibiotic occurred in rice plants by cellular components. Therefore, the stability of the antibiotic in the rice plant extracts was tested *in vitro*. As shown in Table 4, no inactivation of the antibiotic was observed.

Consequently, the phenomenon was considered to be due to the water insolubility of the antibiotic and/or permeability to the rice plant and the organisms.

Antiviral Activity against TMV

Four-week old seedlings of *Nicotiana tabacum* var. Xanthi. were used as the test plants and the coleoptile of 2-week old seedlings of *Phaseolus vulgaris* L., Pinto, were used for the local lesion test. Leaf discs (radius 11 mm) of *Nicotiana tabacum* L. var. Blight yellow were used for disc-assay.

As shown in Table 5, when leaf discs which were prepared immediately before inoculation were floated on various concentrations of the antibiotic in 40 mm petri dishes under 3,000 lux fluorescent illumination at 25°C, the multiplication of TMV in the discs was significantly inhibited when the antibiotic concentration exceeded 1 mcg/ml.

Table 5. Protection of tabacco mosaic virus with aabomycin A

Materials	Concentration (mcg/ml)	Protective * value (%)	Phytotoxicity**
Aabomycin A	100.0	99.8	—
	10.0	87.0	—
	1.0	82.0	—
Blasticidin S	0.01	86.0	+
	0.005	87.0	±
	0.001	82.0	—

* Protective value $= \left(1 - \frac{\text{virus titer (antibiotic-treated leaf)}}{\text{virus titer (antibiotic-untreated leaf)}}\right) \times 100$

** —: no phytotoxicity, ±: slight phytotoxicity, +: low phytotoxicity.

Table 6. Effect of aabomycin A on TMV multiplication in tobacco plant, *in vivo*

Concentration of aabomycin A (ppm)	Post-inoculation time (hour)				
	0	24	48	72	96
1	15 *	52	74	75	61
10	61	89	92	88	80
100	82	95	98	97	89

Control value (*) $= \left(1 - \frac{\text{titer of TNV content in aabomycin-treated seedling}}{\text{titer of TMV content in untreated seedling}}\right) \times 100$

In the *in vivo* test, the antibiotic was sprayed on the seedlings of *Nicotiana tabacum* var. Xanthi, immediately after the inoculation of TMV crude extract. The results shown in Table 6 suggest that the antibiotic inhibited TMV multiplication in the tobacco plant at the concentrations less than 100 ppm. No phytotoxicity was evident even at a concentration of 1,000 ppm. Furthermore, when the antibiotic was sprayed on test plants, 2 days before inoculation, the inhibitory effect of the antibiotic increased. This indicated that the protective effect was higher than curative effect.

Discussion

Many antibiotics have been reported as TMV inhibitors. However, because of their low activities or high phytotoxicities, there is no substance suitable for the practical plant virus disease control use as yet. Though the authors previously reported that laurusin[5] and bihoromycin[6] remarkably inhibited the TMV local lesion formation on pinto bean and TMV multiplication on tobacco leaves, these antibiotics were not satisfactory for use because of their high phytotoxicities. From this view point, the authors found that aabomycin was not only highly effective on TMV multiplication, *in vitro* and *in vivo*, but also less phytotoxic to both rice plants and tobacco, even at the concentration up to 1,000 ppm. Aabomycin A inhibited rice blast disease at 40~80 ppm in pot tests, whereas it exhibited inhibitory effect on the growth of *P. oryzae* at 1.0 mcg/ml *in vitro*.

As previously mentioned, aabomycin A is a water-insoluble substance, and this property might cause lower penetration of the antibiotic into plants. Therefore, the authors now intend to increase its water solubility so as to enhance its activity against the rice blast fungus and TMV.

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