EVALUATION OF MYCOBACILLIN AND VERSICOLIN AS AGRICULTURAL FUNGICIDES

I. ANTIFUNGAL SPECTRUM AND PHYTOTOXICITY

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Two antifungal antibiotics, mycobacillin and versicolin, were studied as agricultural fungicides in the control of fungal infection of rice and jute. Mycobacillin is especially active against *Piricularia oryzae* at a concentration of 10 μg/ml, and versicolin against *Colletotrichum gloeosporioides* at a concentration of 2.5 μg/ml. Mycobacillin has no adverse effect on germination of seeds and growth of seedlings of rice and jute plants at a concentration of 500 ppm, even for prolonged exposure (24 or 48 hours); in fact, it is stimulatory. On the other hand, versicolin has showed detectable phytotoxicity at 500 ppm for prolonged exposure.

Recently, many antifungal antibiotics have been reported. Some of them are also used as agricultural fungicides: blasticidin S[1], kasugamycin[2], polyoxins[3] and validamycin[4]. These antibiotics are effective mainly against the rice blast disease caused by *Piricularia oryzea*; they also improve the condition of morbid garden plants or fruit trees infected by *Sclerotinia* sp. or *Botrytis* sp. Another antifungal antibiotic, griseofulvin, has been used in the past as an agricultural fungicide[5]; it is now given orally to fight dermatophytosis in humans and animals[6]. A new antibiotic, aabomycin A, exhibits marked inhibition of spore germination, mycelial growth, and sporulation of the rice pathogen, *Piricularia oryzae*[7].

Since the late forties, our laboratory has been engaged in searching for new antifungal antibiotics. As a result, two antifungal antibiotics, mycobacillin[8] and versicolin[9] have been isolated. Mycobacillin is a cyclic peptide antibiotic consisting of 13 residues of 7 different amino acids whose structure and mode of action have been reported earlier[10~13]. A tentative structure of versicolin has also been suggested[14]. Both antibiotics show a fairly broad spectrum of activity, particularly the former which inhibits skin pathogens, plant pathogens and saprophytic fungi; it is greatly inactivated in the presence of serum, which limits its use in humans and animals[15]. Versicolin is mainly active against *Trichophyton rubrum* at a dose as low as 1.2 μg/ml. The evaluation of versicolin as an antifungal drug showed that the oral LD_{50} value in mice is 330 mg/kg body weight; in experimental dermatophytosis it cures guinea pigs infected by *Trichophyton rubrum* even at an oral dose as low as 2.5 mg/kg body weight. It has no subacute toxicity in the therapeutic dose range (communicated).

In search of newer uses for these antibiotics, attention was drawn to their possible application in the control of fungal infections of useful agricultural crops, such as rice and jute. The present paper deals with the antimicrobial spectrum and the minimal inhibitory concentration as well as the phytotoxicity of these antibiotics against jute and rice plants.

Materials and Methods

Mycobacillin was isolated from the culture filtrate of *Bacillus subtilis* B_3 according to the method of MAJUMDER and BOSE[16].

Versicolin was isolated from the culture filtrate of *Aspergillus versicolor* N_5 by a method previously reported[17].

Determination of antimicrobial spectrum and minimal inhibitory concentration

The antimicrobial spectra of *B. subtilis* B_3 and *A. vericolor* N_5 were determined by agar cross-streak technique using the producer organisms and test organisms. The media employed were CZAPEK-DOX medium enriched with 0.1 % peptone for jute and rice pathogens and nutrient medium with 1 % peptone for *Xanthomonas oryzae*. These media were selected because *A. versicolor* N_5 does not produce the antibiotic in a medium that does not contain peptone.

The minimal inhibitory concentration of mycobacillin and versicolin against rice and jute pathogenic fungi was determined by agar dilution method. An alcoholic solution of the antibiotic was added to potato-dextrose agar to give various concentrations. Plates were inoculated with pathogenic fungi and results expressed as the minimal concentration required for total inhibition of fungal growth.

Phytotoxicity of mycobacillin and versicolin in terms of germination of rice and jute seeds

Rice seeds: Jaya and Cavery from Rice Research Station, Chinsurah, Hooghly, W. B., India.

Jute seeds: JRO-632 and JRC-212 from Jute Agricultural Research Institute, Nilgung, Barrackpore, W. B., India.

Seeds were washed with sterile water and transferred into a solution of 0.1 % mercuric chloride for 2 minutes. Mercuric chloride solution was then removed by repeated washing with sterile water and seeds were steeped in sterile water for 24 hours. The seeds so treated were transferred into solutions of different concentrations of mycobacillin and versicolin. After given exposures, seeds were taken out and placed into germination plates. The plates were incubated at 30°C in a humidified chamber (relative humidity 100 %) for 5 days in the case of rice seeds and 2 days in the case of jute seeds. Germination was indicated by the formation of root and shoot.

Mycobacillin was dissolved in 0.1 N Na_2CO_3, whereas versicolin was used as an aqueous solution. In control plates, seeds were soaked in 0.1 N Na_2CO_3 or sterile water.

Phytotoxicity of mycobacillin and versicolin in terms of root and shoot length of germinated seeds

Seeds were germinated under normal conditions. The germinated seeds were treated with solutions of mycobacillin or versicolin of different concentrations for various periods of time. In the control experiments germinated seeds were treated with 0.1 N Na_2CO_3 or sterile water. The treated seeds were then placed in sterile petri dishes and incubated at 30°C in a humidified chamber (relative humidity 100 %) for 4~5 days in the case of rice and 3~4 days for jute. The root and shoot lengths were measured (average of 10 seeds) and the results were compared with the control.

Results and Discussion

Antimicrobial Spectrum and Minimal Inhibitory Concentration

Both antibiotic-producing organisms, *B. subtilis* B_3 and *A. versicolor* N_5, are active against rice and jute fungal pathogens (Table 1). But, they are inactive against a very common bacterial rice pathogen, *X. oryzae*.

Mycobacillin is especially active against *P. oryzae*, a rice pathogen, at a concentration of 10 μg/ml, and versicolin against *C. gloeosporioides*, a jute pathogen, at a concentration of

Table 1. Antimicrobial spectrum of *B. subtilis* B_3 and *A. versicolor* N_5 by the agar cross-streak method

Test organism		Activity* of	
		B. subtilis B_3	*A. versicolor* N_5
1. Rice pathogens	*Helminthosporium oryzae*	+	+
	Piricularia oryzae	+	+
	Sclerotium oryzae	−	−
	Xanthomonas oryzae	−	−
2. Jute pathogens	*Macrophomina phaseoli*	+	+
	Botryodiplodia sp.	+	+
	*Colletotrichum corchorum***	+	+
	Colletotrichum gloeosporioides	+	+
	Sclerotium rolfsii	−	−

* symbols: (−) absence of activity, (+) presence of activity
** Isolated by IKATA and TANAKA, 1941

Table 2. Antifungal activity *in vitro* of mycobacillin or versicolin by the agar dilution method

Test organism	Minimal inhibitory conc. (μg/ml)	
	Mycobacillin	Versicolin
Helminthosporium oryzae	50	50
Piricularia oryzae	10	40
Macrophomina phaseoli	50	50
Colletotrichum corchorum	50	25
Colletotrichum gloeosporioides	20	2.5
Botryodiplodia sp.	30	50

0.1 ml of mycobacillin or versicolin was dissolved in a 50% alcoholic solution.

2.5 μg/ml (Table 2). Mycobacillin is of special interest because of its specific action against a very common rice pathogenic fungus, *P. oryzae*, which causes blast disease in rice in Eastern India.

Phytotoxicity of Mycobacillin and Versicolin

Mycobacillin does not inhibit germination of seeds even for a long exposure of 2 days (Table 3). But, versicolin inhibits germination at a concentration of 500 ppm for a long exposure of 24 or 48 hours, although this concentration of versicolin, *i.e.* 500 ppm, has no toxic effect for a short exposure of 2 hours.

Similarly, mycobacillin has no adverse effect on the growth of germinated seeds measured in terms of root and shoot length (Table 4) at a concentration of 500 ppm for short and long exposures. But, in the case of versicolin, some toxic effect at 500 ppm, for long exposures 24 or 48 hours, was observed, short exposure at the same concentration had no such effect.

While studying the phytotoxicity of mycobacillin against rice and jute seeds, we observed that mycobacillin not only stimulates germination but also enhances the growth of root or shoot of germinated seeds (Tables 3 and 4). Mycobacillin-treated seeds germinated to the

Table 3. Phytotoxicity of mycobacillin and versicolin in terms of germination of seeds

Seeds	Time of exposure	Percentage of germination of seeds							
		Control	Treatment: mycobacillin in ppm			Control	Treatment: versicolin in ppm		
			125	250	500		125	250	500
Rice (Cavery)	60 min.	32	35	36	56	60	60	65	62
	120 min.	45.4	50	50	58	62	62	60	60
	24 hr.	51	50	55	65	60	66	46	4
	48 hr.	52	50	59	69	60	56	42	8
Rice (Jaya)	60 min.	50	52	60	60	60	60	55	60
	120 min.	50	69	60	70	60	68	70	70
	24 hr.	64	66	66	76	79	66	48	10
	48 hr.	51	52	69	73	79	66	48	8
Jute (JRC-212)	60 min.	75	75	75	85	75	75	75	75
	120 min.	75	75	75	89	75	75	70	50
	24 hr.	76	70	71	86	79	69	63	57
	48 hr.	84	79	83	88	71	73	71	58
Jute (JRO-632)	60 min.	70	70	75	89	70	70	70	70
	120 min.	70	70	76	88	70	80	65	50
	24 hr.	76	70	75	85	60	69	63	58
	48 hr.	80	80	82	89	70	73	71	57

Table 4. Phytotoxicity of mycobacillin or versicolin in terms of root and shoot length of germinated seeds

Seeds germinated	Time of exposure	Ratio of root to shoot length of germinated seeds							
		Control	Treatment: mycobacillin in ppm			Control	Treatment: versicolin in ppm		
			125	250	500		125	250	500
Rice (Jaya)	60 min.	1/1	1/1.3	1/1.3	1/1.3	1/1	1/1	1/1.2	1/1.2
	120 min.	1/1	1/1.3	1/1.5	1/1.5	1/1	1/1	1/1.2	1/1.2
	24 hr.	1/1.1	1/1.2	1/1	1/1.2	1/1.1	1/1.1	1/1.1	1/1.1
	48 hr.	1/1.1	1/1	1/1	1/1.3	1/1.1	1/1.1	1/1.1	1/1.1
Jute (JRC-212)	60 min.	1/1.3	1/1.3	1/1.4	1/1.4	1/1.3	1/1.1	1/1	1/1
	120 min.	1/1.4	1/1.4	1/1.4	1/1.4	1/1.2	1/1.2	1/1.2	1/1
	24 hr.	1/1.2	1/1.2	1/1.4	1/1.5	1/1.2	1/1	1/0.8	1/0.8
	48 hr.	1/1.5	1/1.9	1/2.9	1/2.5	1/1.5	1/1	1/1	1/0.8

extent of 66~70 %, whereas the control yielded 45~50 %. The ratio of root to shoot length was roughly 1 : 1 or 1 : 1.5 during the first 48 hours in control experiments, whereas in mycobacillin-treated plants the ratio turned out to be 1:1.3 or 1:2, which shows that mycobacillin enhances the growth of both root and shoot, preferentially the latter.

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