INHIBITION OF HYPHAL EXTENSION FACTOR FORMATION BY VALIDAMYCIN IN *RHIZOCTONIA SOLANI*

Sir:

Validamycin (VM) inhibited specifically the hyphal extension of *Rhizoctonia solani* without growth inhibition.[1,2] It was also reported that the hyphal extension factor (HE-factor) which stimulates the hyphal extension of *R. solani*, exists in the hyphae of *R. solani*.[3] The action of VM was antagonized and the extension of VM-inhibited hyphae was stimulated by HE-factor.[4] In spite of the existence of HE-factor in the normal hyphae, the hyphal extension of *R. solani* was inhibited by VM. Consequently, the action of VM was assumed to inhibit the action of HE-factor or to inhibit the formation of HE-factor. In order to examine this problem, following experiments were carried out.

R. solani was grown in 200-ml Erlenmeyer flasks containing 50 ml of medium S (glucose 2%, starch 3%, corn steep liquor 1%, soybean flour 1%, peptone 0.5%, NaCl 0.3%, $CaCO_3$ 0.5%) on a rotary shaker at 28°C for 3 days. The resulting seed culture was inoculated into 200-ml Erlenmeyer flasks containing 50 ml of modified Czapek medium (glucose 3%, peptone 0.2%, $NaNO_3$ 0.2%, K_2HPO_4 0.1%, KCl 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, $FeSO_4 \cdot 7H_2O$ 0.001%) with or without VM (final concentration, 10 μg/ml) on a rotary shaker at 28°C for 3 days. The resulting cultures (750 ml) were filtered and the wet hyphal cake of 29 g or 30 g was obtained from the VM-added culture or VM-free culture, respectively. According to the isolation method of HE-factor[4], both hyphal cakes were extracted with 5 volumes (v/w) of 50% MeOH under reflux for 1 hour. After filtration, the methanol extract of 146 ml or 148 ml was obtained from the VM-added culture or VM-free culture, respectively. After concentration to 50 ml, both extracts were passed through Amberlite IR-120 B columns (H^+, 1×20 cm) to remove VM and then adsorbed on Amberlite IR-410 columns (OH^-, 1×20 cm). After washing with water, both Amberlite IR-410 columns were eluted with 0.2 N NH_4Cl and both elutes were concentrated *in vacuo* to give each HE-factor fraction of 10 ml. The HE-factor fraction of VM-free or VM-added culture is, hereafter, refered to as HE-factor fraction F or V, respectively. The activities of resultant HE-factor fractions F and V were examined by dendroid test method.[1,4]

An agar disk (inoculum) of *R. solani*, which had previously been dipped in a 10 μg/ml solution of VM, was inoculated on a water agar plate containing each HE-factor fraction. Comparing the growth of VM-untreated (normal) or VM-treated (control) inoculum as shown in Table 1, the HE-factor fraction F was found to antagonize the VM inhibition at the 10- or 20-fold dilution after incubation of 2 or 3 days of *R. solani* and the inhibition reversal was increased in response to the concentration of HE-factor.

On the contrary, no reversal of VM inhibition was observed even at the 10-fold dilution of HE-factor fraction V. In order to confirm the absence of contaminating VM in the HE-factor fraction V, the effect of the HE-factor fraction V on the normal hyphae (VM-untreated) was examined. As a result, no inhibition was observed even at the 5-fold dilution of the HE-factor fraction V and hence no contamination of VM was shown in the HE-factor fraction V. No reversal of VM inhibition by the HE-factor fraction V was found not to be due to the action of contaminating VM. Fig. 1 shows the HE-factor activities of HE-

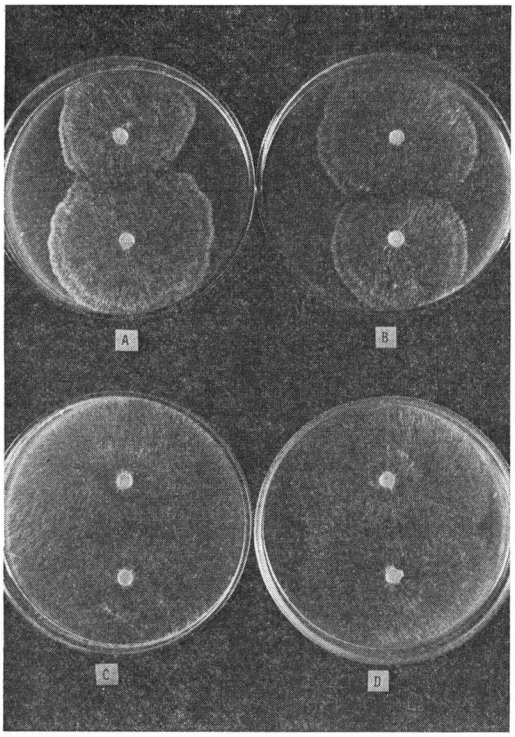
Table 1. Antagonistic activities of HE-factor fractions F and V.

	Time	Growth zone diameter (mm)					
		None (Control)	×10	×20	×50	×100	Normal (VM-untreated)
Fraction F	1 day	18, 17	36, 40	36, 36	32, 32	27, 24	40, 39
	2 day	25, 26	60, 64	48, 47	40, 42	36, 30	69, 68
	3 day	30, 31	>90, >90	66, 66	52, 54	46, 42	>90, >90
Fraction V	1 day	20, 20	36, 37	33, 30	30, 30	26, 28	37, 41
	2 day	26, 26	46, 42	40, 36	36, 36	32, 36	67, 70
	3 day	32, 30	58, 56	48, 52	44, 46	39, 42	>90, >90

Fig. 1. Reversal of VM-inhibition by HE-factor fraction V or F.

A, B : 10- or 20-fold dilution of HE-factor fraction V, respectively.

C, D : 10- or 20-fold dilution of HE-factor fraction F, respectively.



factor fractions F and V. In another experiment using VM as a final concentration of 50 μg/ml, the inhibition of HE-factor formation was also shown in a similar manner.

Thus, the formation of HE-factor in the VM-added culture was considered to be inhibited by VM.

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