バリダマイシンAのイネ紋枯病菌および数種菌核病菌のトレハラーゼに対する効果

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
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巻/号	55巻2号
掲載ページ	p. 238-241
発行年月	1989年4月

日植病報 55: 238–241 (1989)
Ann. Phytopath. Soc. Japan 55: 238–241 (1989)

短　報
Phytopathological Note

Effect of Validamycin A on the Activity of Trehalase of *Rhizoctonia solani* and Several Sclerotial Fungi*

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Key words: validamycin A, trehalase, *Rhizoctonia solani*, sclerotial fungi.

Validamycin A (VM-A), a pseudo-oligosaccharide produced by *Streptomyces hygroscopicus* var. *limoneus*, is effective for the control of sheath blight of rice caused by *Rhizoctonia solani* AG-1[1]. VM-A is also effective to control other crop diseases caused by sclerotial fungi such as *R. solani* AG-2-2, *R. oryzae, Sclerotium fumigatum, S. oryzae-sativae* and *S. hydrophilum* as well as sheath blight caused by *R. solani* AG-1[2]. VM-A is known to cause growth retardation and abnormal branching of hyphae in water agar and inhibit the formation of infection cushion of *R. solani* AG-1[3]. As for the mode of action of VM-A, several works have been reported[1,4–6]. VM-A had potent inhibitory activity against trehalase of *R. solani* AG-1, without giving any significant inhibitory effect against any other glycohydrolase enzymes tested[6]. Trehalose is well-known as a storage carbohydrate and trehalase plays an essential role in the transport of D-glucose in fungi[7–9].

In the present paper we show that VM-A inhibits the activity of trehalases obtained from several sclerotial fungi as well as that from *R. solani* AG-1.

Fungi. *R. solani* Kühn AG-1 (strain TKF-44), *R. solani* Kühn AG-2-2 (TKF-303), *R. oryzae* Ryker et Gooch (TKF-206), *S. fumigatum* Nakata et Kawamura (TKF-203), *S. oryzae-sativae* Sawada (TKF-201) and *S. hydrophilum* Saccardo (TKF-145) were used.

Preparation of trehalase. The crude trehalase was prepared as described by Asano *et al.*[6] with a slight modification. Mycelial agar blocks of each fungus obtained from the advancing edge of the two- or three-day culture growing on potato sucrose agar were inoculated to potato sucrose liquid medium and incubated for 4 days at 27 C on a reciprocating shaker. Mycelia were harvested onto filter paper by vacuum filtration and washed with deionized water (DW) several times and finally with 10 mM phosphate buffer (pH 6.0). The washed mycelia were stored at −20 C until use without losing trehalase activity. Mycelia (10 g in wet weight) were homogenized with 10 mM phosphate buffer in a Waring Blender for 5 min. The homogenate was centrifuged at 4,000×*g* for 20 min. The precipitate was further homogenized together with dry ice using a mortar and pestle for 40 min. The cold powder obtained was suspended in DW and disrupted with supersonic oscillator (15 kV) under cooling for 30 min. Then

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* The outline of this paper was reported in August of 1988 at 5th International Congress of Plant Pathology in Kyoto, Japan.

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this suspension was centrifuged at 4,000×*g* for 20 min. The precipitate was suspended in a small amount of DW and dialyzed against DW at 4 C overnight. The total volume of this suspension was adjusted to 20 ml with DW and the aliquots were used as the crude enzyme solution.

Assay of trehalase activity. Trehalase activity was measured by the method based on the colorimetric determination of glucose released from trehalose[6]. The reaction mixture consisted of 125 μl of enzyme solution, 50 μl of 0.4 M trehalose, 20 μl of VM-A (Takeda Chemical Industries Ltd., 91.3% of purity) or DW and 305 μl of 0.1 M phosphate buffer (pH 6.0). After incubation in a microtest tube (1.5 ml) for 30 min at 37 C in a water bath, the tube was immersed for 10 min in boiling water to stop the reaction and then cooled. The solution was centrifuged at 4,000×*g* for 5 min and 20 μl of the supernatant was added to the Glucose B-Test Wako reagents (Wako Pure Chemical Industries Ltd., 3 ml) containing glucose oxidase, peroxidase, phenol and 4-aminoantipyrine. After incubation for 20 min at 37 C in a water bath the amount of released glucose was determined by measuring the optical density at 505 nm with a spectrophotometer.

The activity of trehalase in sclerotial fungi. In all the sclerotial fungi used in this study, most of the activity of trehalases was detected in the sedimented cell debris fraction of mycelial homogenate obtained as described above, although not a negligible amount of trehalase activity was also detected in the supernatant fraction. Treatment of cell debris fraction with Triton X-100 (1%) did not decrease the trehalase activity of this fraction, suggesting that trehalases in the cell debris fraction are firmly bound to cell wall. The activity of trehalase per 1 g of wet mycelia varied depending on fungal sources (Fig. 1). The rate of glucose released from trehalose by trehalase of *R. solani* AG-1 and *S. fumigatum* were about 20-fold of that by trehalase of *R. oryzae* (Fig. 1). In the experimental condition used in this study, each trehalase activity

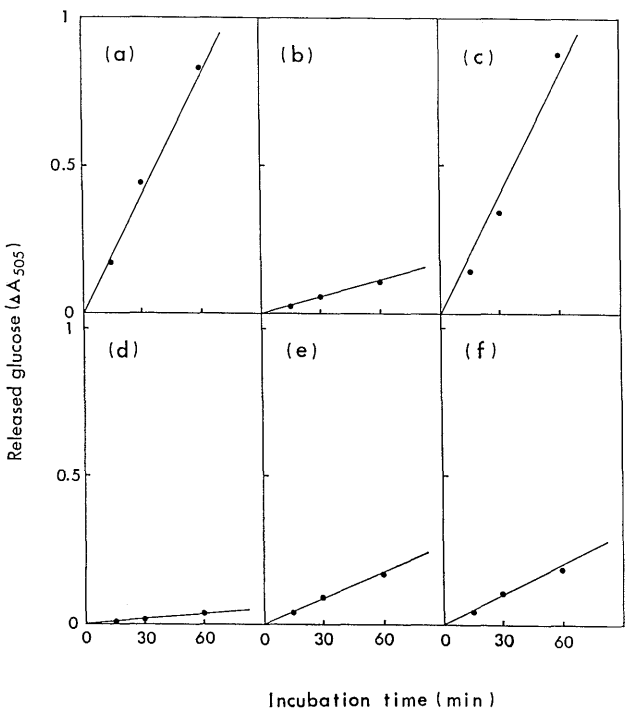

Fig. 1. Trehalase activity of *Rhizoctonia solani* AG-1 (a), *R. solani* AG-2-2 (b), *Sclerotium fumigatum* (c), *R. oryzae* (d), *S. oryzae-sativae* (e) and *S. hydrophilum* (f). Trehalase activity was determined by the amount of released glucose (ΔA_{505}).

was kept constant at least for 1 hr of incubation as apparent by the linear increase of released glucose with time (Fig. 1).

The inhibition of trehalase activity by VM-A. All trehalases obtained from six different fungi used here were inhibited by VM-A. The concentration of VM-A required to give 50% inhibition (IC_{50}) of trehalase activity was between 3.4×10^{-6} M to 1.8×10^{-5} M (Table 1). The inhibition behavior of VM-A against trehalases of *R. solani* and *S. fumigatum* was examined by Lineweaver-Burk plot. The results indicated that VM-A was a competitive inhibitor against trehalase of *R. solani* and *S. fumigatum*. The K_i value of VM-A was about 5×10^{-6} M where the K_m value of trehalase was about 3×10^{-3} M in *R. solani* AG-1 and the K_i value was about 1×10^{-5} M where the K_m value was about 1×10^{-2} M in *S. fumigatum* (Fig. 2). A similar result was reported in the inhibition behavior of validoxylamin A which also inhibits the activity of trehalase of *R. solani*[6].

In this paper we showed that VM-A inhibited the activity of trehalase in sclerotial fungi as well as in *R. solani* AG-1. This suggests that the inhibition of trehalase may be a common mode of action of VM-A in controlling these sclerotial diseases.

We wish to thank Dr. Y. Kameda, University of Hokuriku, for his suggestion and criticism.

Table 1. Concentration of VM-A required to give 50% inhibition (IC_{50}) of trehalase activity in sclerotial fungi

Source of trehalases	IC_{50} (μM)
Rhizoctonia solani (AG-1)	4.6
Rhizoctonia solani (AG-2-2)	18.0
Rhizoctonia oryzae	11.0
Sclerotium fumigatum	7.6
Sclerotium oryzae-sativae	3.4
Sclerotium hydrophilum	7.2

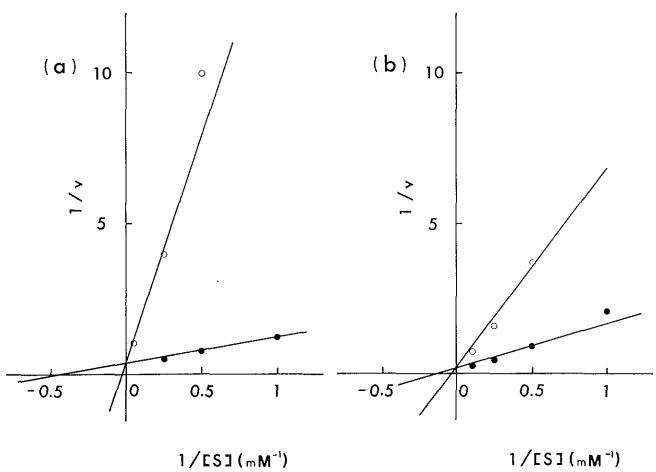

Fig. 2. Lineweaver-Burk plots for the inhibition of trehalase by VM-A in *Rhizoctonia solani* AG-1 (a) and *Sclerotium fumigatum* (b). Rate of reaction was represented by $\triangle A_{505}$. Without VM-A (●), with 5×10^{-6} M of VM-A (○), (b) with 10^{-6} M of VM-A (○).

和 文 摘 要

繁本令子・奥野哲郎・松浦一穂：バリダマイシンAのイネ紋枯病菌および数種菌核病菌のトレハラーゼに対する効果

イネ紋枯病菌および数種菌核病菌には細胞壁画分にトレハラーゼが存在した。そしてバリダマイシンA (VM-A) はこれらすべてのトレハラーゼ活性を阻害した。また，VM-A のトレハラーゼに対する 50% 活性阻害濃度はそれぞれ 3.4×10^{-6} M から 1.8×10^{-5} M であった。Lineweaver-Burk の逆数プロットよりトレハラーゼに対する VM-A の阻害形式を調べると拮抗型であった。これらの結果より，イネ数種菌核病菌に対する VM-A の効果は紋枯病菌の場合と同じくトレハラーゼ活性阻害によるものと考えられる。

(Received October 27, 1988)