Note

Effect of Validamycin on β -D-Glucan-degrading Enzymes from *Rhizoctonia solani*

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Validamycin,¹⁾ an aminoglycoside antibiotic, alters the morphology of *R. solani* without inhibiting its growth. That is, the antibiotic inhibits the extension of the main hyphae whereas there is almost no inhibition of the primary or secondary branches.^{2,3)} It was reported that validamycin also affected the component sugars and linkages of the cell wall glycan of *R. solani*,⁴⁾ and the production of laminarinase and glucan synthetase by the fungus.⁵⁾ In the presence of validamycin, the production of laminarinase increased in the culture filtrate and decreased in the mycelium, while the intracellular production of glucan synthetase increased to some extent.⁵⁾

β -D-Glucosidase and β -1,3-glucanase are thought to be the main enzymes involved in the degradation of laminarin. To clarify the effects of validamycin on these enzymes, the activity of each was examined as follows. For the β -D-glucosidase assay, the assay mixture comprised 50 mM sodium acetate buffer (pH 5.5), a given amount of enzyme solution and 4 mg of salicin in a total volume of 2 ml. For the β -1,3-glucanase assay, the assay mixture comprised the same buffer, a given amount of enzyme solution and 250 μ g of chlorhexidine. Other procedures were identical to those for the laminarinase assay described in a previous paper.⁵⁾ The optimum conditions for the β -glucosidase were examined using the culture filtrate after 6 days culture. The maximum activity was found at pH 5 and 65°C. However, the enzyme assay was performed at pH 5.5 and 50°C in order to adjust the experimental conditions to those for the previously reported laminarinase assay.⁵⁾

As previously described,⁵⁾ *R. solani* was cultured in the presence of various concentrations of validamycin and then the enzyme activities in the filtrates were assayed. The results are shown in Table I. It was found that the production of the β -glucosidase remarkably increased with more than 10 μ g/ml of validamycin. The ratio of increase was about 60% in this case, but in another instance the enzyme production in the culture with validamycin (10 μ g/

ml) increased by more than 100%, compared with that in the control culture. To assay only the β -1,3-glucanase activity, it was necessary to perform the assay with pachyman as the substrate with inhibition of the β -D-glucosidase activity. Among various metal ions and inhibitors, Hg^{2+} , DFP and chlorhexidine markedly inhibited the β -D-glucosidase activity. The inhibition by chlorhexidine was distinguishable from that of the others in the case of salicin as the substrate. Hereafter, the β -1,3-glucanase assay was performed with pachyman as the

TABLE I. EFFECT OF VALIDAMYCIN ON THE EXTRACELLULAR PRODUCTION OF β -D-GLUCOSIDASE

Cultivation time (days)	VM in medium (μ g/ml)	β -D-Glucosidase activity*	Relative activity (%)
6	0	2.11	100.0
	1	2.12	100.5
	10	3.25	158.6
	50	3.23	153.1
9	0	2.06	100.0
	1	2.35	114.1
	10	3.17	153.9
	50	3.50	169.9

VM, validamycin.

* OD at 600 nm/ml.

FIG. 1. Time Course Changes of the Intracellular Production of β -D-Glucan-degrading Enzymes.

Enzyme activities in a culture without (open symbols) or with (closed symbols) 10 μ g/ml of validamycin were assayed with pachyman, ○, ●; laminarin, △, ▲; and salicin, □, ■, as substrates, respectively.

substrate in the presence of 250 μ g of chlorhexidine. As a result, it was clarified that the extracellular production of β -1,3-glucanase slightly decreased in the presence of validamycin. The ratio of decrease was less than 20%.

Figure 1 shows the time course changes of the intracellular production of β -D-glucan-degrading enzymes. In the control culture, the enzyme activities rose until 8 days culture regardless of the kinds of substrates. While, in the cultures with validamycin, all the activities markedly decreased after 2 days culture. These results clarified that the intracellular production of β -D-glucosidase decreased in the presence of validamycin. The ratio of decrease was as much as 70 to 80%. Furthermore, the intracellular production of β -D-glucan-degrading enzymes other than β -D-glucosidase may decrease in the presence of validamycin. With regard to β -1,3-glucanase, the assay was performed in the same way as with the culture filtrate. The effect of validamycin on the intracellular production of β -D-glucan-degrading enzymes (with pachyman as the substrate) was not noticeably influenced by chlorhexidine. From the results, the intracellular production of β -1,3-glucanase was confirmed to decrease remarkably in the presence of validamycin.

It was also investigated as to whether or not validamycin affected the activities themselves of β -D-glucan-degrading enzymes (determined with pachyman, laminarin or salicin as the substrate) during incubations. With any substrate, these enzyme activities in the presence of validamycin (even with 500 μ g) were almost the same as those in the control, and thus the results indicate that validamycin never affects the activities themselves of β -D-glucan-degrading enzymes.

From the facts mentioned above, the following was clarified: the main enzymes involved in the degradation of laminarin were β -1,3-glucanase and β -D-glucosidase. Although validamycin had no effect on the activities themselves of these enzymes, the antibiotic significantly affected their production. The intracellular production of β -1,3-glucanase remarkably decreased in the presence of validamycin. If the enzyme in the mycelium participates in the modification of cell wall glycan, the ratio of β -1,3-glycosyl residues in the cell wall glycan may as a result increase in the presence of validamycin, since the enzyme production was inhibited by the antibiotic. We are now

investigating the structure of the cell wall glycan, with or without validamycin.

The intracellular production of β -D-glucosidase remarkably decreased, while its extracellular production increased in the presence of validamycin. This suggests that the role of β -D-glucosidase in the mycelium may be different from that in the culture filtrate. Perhaps the enzyme in the mycelium participates in the regulation of the building up of new sugar chains. Then, the decrease in the intracellular production of the enzyme may induce the creation of new cell wall polysaccharides. Several investigators have reported a close relation between endogenous β -D-glucan-degrading enzymes and the cell wall constitution or morphogenesis of the fungus.⁶⁻⁸⁾ The results of the present study suggest that the decrease in the production of these enzymes may stimulate the synthesis of cell wall polymers, and this stimulation may cause morphological changes, although no definite evidence has been obtained yet of the relationship of these enzymes to the morphological changes of *R. solani* in the presence of validamycin.

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