Studies on the Control of Plant Diseases by Amino Acid Derivatives

(3) Effect of sodium N-lauroyl-valinate on the growth of rice blast fungus

Yasuo HOMMA*, Tetsuo NAKAJIMA**, Toshiro SHIDA***,
and Tomomasa MISATO*

本間保男*・中島哲男**・志田俊郎***・見里朝正*: アミノ酸関連
物質による植物病害の防除 (3) イネいもち病菌の生育に
及ぼす sodium N-lauroylvalinate の影響

Abstract

As described in the previous paper, it was clear that N-lauroyl-L-valine had protective effect on rice blast. Experiments were conducted to examine the fungicidal activity of this compound on each stage of the fungal life cycle, *i. e.* spore germination, appressorial formation, hyphal penetration, hyphal growth, and sporulation, and to compare the activities between optical isomers. The results indicated sodium N-lauroyl-L-valinate inhibits almost completely the appressorial formation at 100 $\mu\text{g/ml}$, and the mycelial growth at 200 $\mu\text{g/ml}$. The isomer inhibited the appressorial formation and the mycelial growth above 90 per cent at 300 $\mu\text{g/ml}$, but no other serious inhibition was observed. The DL-mixture inhibited the appressorial formation at 100 $\mu\text{g/ml}$, and showed less inhibitory effect than the L-isomer in other stages.

Thus, appressorial formation was suggested to be one of the main inhibitory sites by N-lauroylvaline isomers in protection against rice blast.

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Introduction

We examined effect of some amino acid derivatives on rice blast disease and found that N-lauroyl derivatives of L-methionine, L-leucine, DL-alanine and L-valine have protective effect on the rice blast disease. N-lauroyl-L-valine and its sodium and potassium salts were active, but other salts and esters were not so effective²⁾.

It has been pointed out that effect of amino acids on plant pathogens varies according to their structure including their optical isomerism^{3,8,10,11)}. Kuè *et al.* indicated that some varieties of apple treated with D- and DL-phenylalanine increased the resistance markedly against the apple scab caused by *Venturia inaequalis*, and showed that with the increase in resistance the conversion of phloridzin to phloretin by β -glucosidase and the oxidative reaction by phenoloxidase occurred^{1,6,7)}.

This paper deals with the effect of optical isomers of sodium N-lauroylvalinate on the growth stages of rice blast fungus, *i. e.* spore germination, appressorial formation, hyphal penetration, hyphal growth and sporulation.

* The Institute of Physical and Chemical Research, Wako, Saitama, Japan. 理化学研究所

** Faculty of Agriculture, Tokyo University of Education, Tokyo, Japan. 東京教育大学

*** Central Research Laboratory, Ajinomoto Inc. Kawasaki, Japan. 味の素株式会社中央研究所

Materials and Methods

1. Inhibition of spore germination

The test fungus was cultured on a yeast starch agar slant for 8 days at 28 C, and spore formed were used for this test. The spores were suspended in 2% sucrose solution, and the number of them was adjusted by microscope observation ($\times 150$) to 30–40 spores *per field of view*. Equal volumes of the spore suspension and the solution containing test compounds were mixed and stirred thoroughly, and one drop of the mixture was placed on a glass slide⁴. After it was kept in a moist chamber for 24 hours, the number of germinated spores was counted.

2. Inhibition of appressorial formation

The spore suspension was dropped on a hole slide glass, and incubated in a moist chamber at 28 C for 4 hours. After mixing equal volumes of the solution containing test chemicals and spore suspension, the mixtures were kept in the moist condition. The observation of appressorial formation was carried out after 90 per cent of untreated spores formed appressoria.

3. Inhibition of hyphal penetration through the artificial membrane

Because hyphal penetration into plant cells is an necessary process in disease development, test for inhibition of this process by the isomers was undertaken, using an artificial membrane, cellophane, as a model of living cell membrane⁹. Solution containing test chemicals and the fresh rice leaf homogenate were poured into a petri dish of 9 cm diameter. Two lumps of sponge ($2 \times 2 \times 1$ cm) were placed in the petri dish and a sheet (1.5×0.5 cm) of cellophane membrane (made by Visking for dialysis) was placed on each sponge.

Sterilized gauze (0.5×0.5 cm) was placed on it so that the spore suspension might not flow out. The spore suspension (0.02 ml) (the number of spores was 15–20 *per field of view at $150 \times$ under a microscope*) was dropped on the gauze. After keeping for 48 hours at 28 C, the number of pores on the cellophane membrane which was caused by hyphal penetration was counted by microscope observation ($\times 280$). Inhibition of hyphal penetration was estimated from the mean value of the 20 observations.

4. Inhibition of hyphal growth

The spore suspension (0.5 ml) prepared by the above method was added to 8.5 ml of yeast-glucose (YG) liquid medium in a test tube and incubated for 48 hours at 28 C in a tube shaker. One ml of solution containing each of the test compounds was added in each tube and cultured for another 48 hours under the same condition. The mycelia were filtered, washed with distilled water and dried up for an hour at 100 C. The growth during the culture was evaluated by the dry weight. Mycelial growth on yeast-starch (YS) agar plate containing test chemical was also evaluated by measuring diameter of the mycelial colony after 10 days culture at 28 C on the plate.

5. Inhibition of sporulation

The spore suspension (0.2 ml) (the number of spores was adjusted to 30–40 *per field of microscopic view ($\times 150$)*) was expanded on the surface of yeast-starch agar slant. After incubating for 4 days at 28 C, 10 ml solution of test chemicals of given concentration was poured to the surface of the slant medium and left for 10 minutes. After removing the chemical solution, culture was continued for another 4 days at 28 C. The inhibitory effect on the sporulation was estimated by the number of spores, which were suspended in 10 ml water per slant and counted with Thoma haemostometer cell.

Results

1. Inhibition of spore germination

The inhibition of spore germination by chemicals is shown in Fig. 1. The isomers did not inhibit

Fig. 1. Effects of isomers of N-lauroylvaline on spore germination.

Fig. 2. Effects of isomers of N-lauroylvaline on appressorial formation.

Fig. 3. Effects of isomers of N-lauroylvaline on hyphal penetration through celophane membrane.

Fig. 4. Effects of isomers of N-lauroylvaline on hyphal growth.

Fig. 5. Effects of isomers of N-lauroylvaline on hyphal growth on YS medium.

Fig. 6. Effects of isomers of N-lauroylvaline on sporulation.

spore germination at the concentration below 200 $\mu\text{g/ml}$. Sodium N-lauroyl-L-valinate inhibited spore germination completely at 500 $\mu\text{g/ml}$. While, the D-isomer raised inhibition with increase in the concentration from 200 $\mu\text{g/ml}$, but at 1,000 $\mu\text{g/ml}$ the inhibition ratio was about 80%. The racemic mixture showed an intermediate inhibition rate between L- and D-isomers, and inhibited spore germination perfectly at 1,000 $\mu\text{g/ml}$.

2. Inhibition of appressorial formation

Sodium N-lauroyl-L-valinate inhibited about 30% of appressorial formation at 50 $\mu\text{g/ml}$. The inhibition was remarkably raised, and reached to 100% at 200 $\mu\text{g/ml}$ (Fig. 2). Sodium N-lauroyl-DL-valinate showed almost the same inhibitory activity as that of L-isomer. Sodium N-lauroyl-D-valinate did not inhibit appressorial formation at 100 $\mu\text{g/ml}$, but the inhibition increased with increase in the concentration, and attained to 100% at $\mu\text{g/ml}$. Slope of the dosage-inhibition curve was almost the same in the three cases, but the curve of D-isomer shifted to higher concentration region as compared with those of the L-isomer and the DL-mixture.

3. Inhibition of hyphal penetration through the artificial membrane

Inhibition of hyphal penetration through a model membrane by the test compounds was shown in Fig. 3. The three samples showed almost equal inhibitory effect. The inhibition of the two isomers and their racemic mixture was about 30% at 50 $\mu\text{g/ml}$, and gradually raised with the increase in concentration, and attained to 100% at 500 $\mu\text{g/ml}$.

4. Inhibition of hyphal growth

Sodium N-lauroyl-L-valinate inhibited 30% of the hyphal growth at the concentration of 50 $\mu\text{g/ml}$, and then, the inhibition was raised with the increase in the concentration, and showed 100% inhibition at 200 $\mu\text{g/ml}$ (Fig. 4). The D-isomer did not inhibit at 100 $\mu\text{g/ml}$, but the inhibition was raised with the increase in the chemical concentration to 100% at 300 $\mu\text{g/ml}$. In inhibition tests of hyphal growth, as well as spore germination, the DL-mixture showed intermediate activities, and attained to 100% inhibition at 300 $\mu\text{g/ml}$.

Inhibition of mycelial growth on YS agar plate was very similar in every cases of D- and L-isomers and DL-mixture (Fig. 5). The isomers inhibited hyphal growth less than 20% at 500 $\mu\text{g/ml}$, and 100% at 1,000 $\mu\text{g/ml}$. It is supposed that the inhibitory effect in shaking culture would be more remarkable than stationary culture in liquid medium. Cultures on agar plate may show the weakest inhibitory effect among the three methods. Therefore, the inhibition of stationary culture in liquid medium may be situated between the shaking culture and the culture on agar plate.

5. Inhibition of sporulation

Sodium N-lauroyl-L-valinate enhanced inhibition of sporulation with the increase in its concentration from 50 $\mu\text{g/ml}$ and inhibited completely at 1,000 $\mu\text{g/ml}$ (Fig. 6). On the other hand, the inhibition of the D-isomer and the DL-mixture was 15% at 50 $\mu\text{g/ml}$, and increased with the increase in the concentration to about 55% at 1,000 $\mu\text{g/ml}$.

Discussion

The results of the experiments were summarized in Fig. 7. There were some differences in the inhibition pattern among three samples. Appressorial formation was the most strongly affected by the L-isomer and the DL-mixture, and hyphal penetration and hyphal growth were also inhibited by them to some extent. The D-isomer inhibited hyphal penetration and appressorial formation to the highest degree, and also hyphal growth to a certain degree. Thus, it may be considered that the primary inhibitory site of the isomers will be at the appressorial formation. As a general rule, the order of inhibitory action among three samples was $L > DL > D$.

Kuč *et al.*³⁾ treated five varieties of apple susceptible to *Venturia inaequalis* with D-, DL-, and L-phenylalanine and they observed that lesions by inoculating the pathogen were remarkably different after the treatment with the respective samples, namely, the degrees of susceptibility decreased in order of L-, DL-, D-phenylalanine, and L-phenylalanine treated apple was equal to untreated apple in the development of the disease.

Ohata *et al.*⁸⁾ examined the difference in inhibitory effect of amino acids isomers, and their result showed that D-aspartic acid and D-leucine had a little inhibition for germ tube growth and did not inhibit the hyphal growth at all, but the L-isomer of both the amino acids did not affect germ tube and hyphal growth of rice blast fungus. Homma *et al.*²⁾ described that L-valine had no preventive effect on rice seedlings.

On the contrary, the isomers of the amino acid derivatives tested in the present study did not always agree with the cases of aspartic acid, leucine, and phenylalanine as stated above. This may be depend on N-acylated structure of the test compounds.

The inhibitory effect on growth of rice blast fungus by the isomers did not correspond with the order of protection against rice blast disease. From the fact that the order of the disease protection was $D \geq L > DL$, which the order of the inhibition tested in the present experiment was $L > DL > D$, it may be assumed that the rice plant treated with the D-isomer increases the resistance to that fungus as Kuč *et al.* described in their paper³⁾. We can not explain clearly the difference of the results between *in vitro* and *in vivo* unless we further analyze the physical and chemical factors of these isomers.

Fig. 7. ED₅₀ value of isomers of N-lauroylvaline for each growth stage of *P. oryzae* N₁.

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和文摘要

アミノ酸関連物質による植物病害の防除

(3) イネいもち病菌の生育に及ぼす Sodium
N-lauroylvalinate の影響

本間 保男・中島 哲男・志田 俊郎・見里 朝正

前報で N-lauroyl-L-valine はいもち病防除効果を有することが明らかとなったので、本剤がいもち病菌の生活史すなわち胞子発芽、付着器形成、菌糸侵入、菌糸伸長ならびに胞子形成などのどの生育期にもっとも作用するかを調べ、さらに D-異性体及び D-, L-体との比較をも試みた。その結果、L-体の Na 塩は付着器形成を 100 μ g/ml で、菌糸伸長を 200 μ g/ml でそれぞれ約 100% 阻害した。また D-体の Na 塩は付着器形成及び菌糸伸長を 300 μ g/ml の濃度でそれぞれ 90% 以上阻害したが、他の生育期には軽い阻害しか示さなかった。さらに DL-体の Na 塩は付着器形成を 100 μ g/ml で約 90% 阻害し、他の生育期ではいずれも L-体より阻害率が劣っていた。したがって N-lauroylvaline 各光学異性体の Na 塩はいずれも付着器形成阻害をもっとも強く、ついで菌糸伸長を阻害した。またこれら異性体の間の阻害率は L>DL>D の順であった。