

Alternaria

alternata群病原菌における宿主特異的毒素生成の制御 (3)

ナシ黒斑病菌野生株における病原性の不安定性

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Metabolic Regulation of Host-Specific Toxin Production in *Alternaria alternata* Pathogens (3). Instability of Pathogenicity in Field Isolates of *A. alternata* Japanese Pear Pathotype*

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Key words : *Alternaria alternata* pathogen, host-specific toxin, metabolic regulation, non-pathogenic isolate.

Host-specific toxins (HSTs) produced by several pathotypes of *Alternaria alternata* are now known to be an essential mediator for the induction of compatibility between the HST producers and their susceptible plants^{1,2)}. However, the field isolates of *A. alternata* pathogens frequently lose their ability to produce HST under laboratory³⁾ or field⁴⁾ conditions, particularly at considerably higher temperatures (*e. g.*, in hot summer); no longer recognizable as a distinct pathotype. Experimental approaches to the mechanism of instability of pathogenicity are essential for understanding the disease epidemiology. This article presents some of fundamental evidence on the concomitant loss of pathogenicity and toxin productivity in *A. alternata* Japanese pear pathotype, the causal fungi of black spot disease of Japanese pear. This pathotype produces AK-toxin as a HST, and is characterized by affecting only certain cultivars of Japanese pear which are comparable to the AK-toxin-sensitive cultivars⁵⁾.

All isolates were obtained by single spore isolation from spore mass formed on lesions. *Alternaria*-infected pear leaves were sampled from two Nijisseiki pear orchards of Nagoya University and Anjo Agricultural High School in May and June, 1983. Ten sampled leaves were used in this experiment; three of them were from Nagoya University (Nagoya lesions) which were under short-term pear cultivation (5 years), and the other seven samples were from Anjo Agricultural High School (Anjo lesions) which were under long-term pear cultivation (*ca.* 50 years). Each lesion on diseased leaves

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1) Nishimura, S. and Kohmoto, K. (1983). In Toxin and Plant Pathogenesis (Daly, J. M. and Deverall, B. J. eds.). Academic Press Australia, Sydney, pp. 137-157. 2) Nishimura, S. and Kohmoto, K. (1983). Ann. Rev. Phytopathol. 21 : 87-116. 3) Nishimura, S., Kohmoto, K., Otani, H., Ramachandran, P. and Tamura, F. (1982). In Plant Infection: The Physiological and Biochemical Basis (Asada, Y., Bushnell, W. R., Ouchi, S. and Vance, C. P. eds.). Japan Sci. Soc. Press and Springer-Verlag, Tokyo and Berlin. pp. 199-213. 4) Tsuge, T. and Nishimura, S. (1984). Ann. Phytopath. Soc. Japan 50 : 189-196. 5) Otani, H., Kohmoto, K., Nishimura, S., Nakashima, T., Ueno, T. and Fukami, H. (1985). *Ibid.* 51 : 285-293.

was cut into *ca.* 2 mm² pieces with sterile scissors, placed in a test tube containing 3 ml sterilized water with 100 ppm streptomycin and shaken for 1 min to suspend the spores of *A. alternata* on the lesions. An aliquot (0.1 ml) of leaf wash was plated onto PDA medium containing 100 ppm streptomycin in a Petri dish (9 cm in diameter). After incubation at 25 C for 2 days, the plates were observed under microscope to confirm the colonies originated from single *A. alternata* spore. The colonies were transferred to test tubes containing 5 ml of Richards' liquid medium and were then cultured at 25 C for 10 days. At the end of incubation period, small piece of each mycelial mat was transferred to a new test tube containing the same medium.

In each step of the successive transfers, the spores that were produced on harvested mycelial mats were collected as described previously⁴⁾. All isolates of *A. alternata* were tested for pathogenicity on the detached young leaves of Nijisseiki pear by using spore suspensions as described previously⁴⁾. Appearance of black necrotic spot within 24 hr of incubation was the criterion used to determine pathogenicity. Nonpathogenic isolates formed no spots within 24 hr of incubation, and all isolates except the nonpathogenic ones formed more than 30 spots per cm² leaf.

A total number of 197 isolates taken from lesions were assayed for their pathogenicity just after single spore isolation. Twenty eight isolates were found to be already nonpathogenic to pear leaves. Of the 197 isolates, one hundred isolates were selected and grouped into ten; each group had ten isolates from one lesion. These isolates were subjected to successive transfers to observe the changes of their pathogenicity (Fig. 1). The nonpathogenic mutants gradually increased in number during the successive transfers.

The appearance of nonpathogenic mutants in ten groups could be classified into four types. Type 1 was indicated by Nagoya lesions 1 and 2. This is a special case where almost all isolates were found to be nonpathogenic just after isolation from diseased lesions. Type 2 was indicated by Nagoya lesion 3; all isolates expressed considerable pathogenicity just after isolation, but the number of nonpathogenic isolates increased immediately after the transfers. Type 3 was in Anjo lesions 1, 2, 3 and 4; almost all isolates were pathogenic just after isolation, but nonpathogenic isolates gradually appeared with the successive transfers. Type 4 was in Anjo lesions 5, 6 and 7; all isolates maintained considerable pathogenicity even after 10 transfers. These results indicate a significant variation in pathogenicity in the two orchards; isolates from Nagoya lesions had higher frequency in the appearance of nonpathogenic mutants when compared with isolates from long-term cultivation in Anjo. The loss of pathogenicity might be related to unstable nature of the pathogen population under short-term field cultivation in the orchard.

None of the nonpathogenic mutants derived from pathogenic isolates during the successive transfers showed intermediate pathogenicity; when pathogenic isolates mutated to nonpathogenic ones, they became completely pathogenicity-less mutants. Such situation of pathogenicity seems to be characteristically associated with AK-toxin, and substantiates the notion that AK-toxin specifically participates in the expression of pathogenicity.

Consequently, the isolates were tested for their loss of AK-toxin productivity. A total of 78 isolates were subjected to the bioassay for toxin production during spore ger-

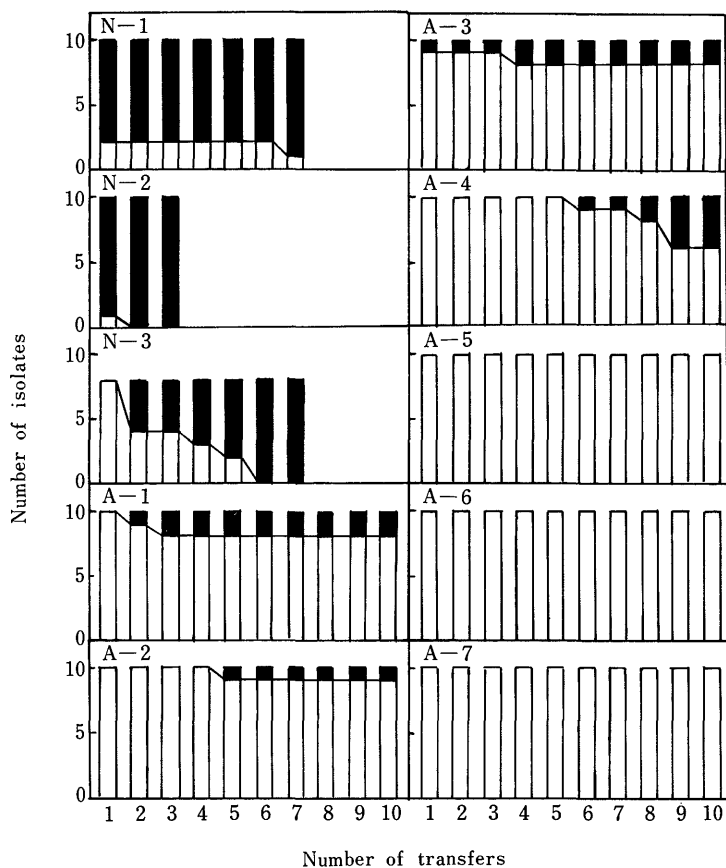


Fig. 1. Changes in pathogenicity of field isolates of *A. alternata* Japanese pear pathotype during successive transfers on Richards' liquid media. Each group of isolates was obtained from spores formed on each lesion by single spore isolation. N (Nagoya lesion)-1, 2 and 3, and A (Anjo lesion)-1, 2, 3, 4, 5, 6 and 7 were sampled from the orchards of Nagoya University and Anjo Agricultural High School, respectively.

□ : pathogenic isolates, ■ : nonpathogenic isolates.

mination. Spore-germinated fluids prepared by placing spore suspension (10^6 spores/ml) on paper towel and harvesting after 24 hr of incubation at 25 C were used for the evaluation of toxin production as described previously⁶). All nonpathogenic isolates did not produce AK-toxin, while all pathogenic isolates produced detectable toxin (Table 1). Thus, the instability of pathogenicity in *A. alternata* pathogens seems to be resulted from the instability of the HST productivity. This result suggests that the HST from germinating spores plays a critical role in the successful pathogenesis.

Mycelia and spores of isolates which lost their pathogenicity were morphologically indistinguishable from those of pathogenic parental isolates. It suggests that they mere-

6) Tsuge, T., Nishimura, S., Omura, S., Kohmoto, K. and Otani, H. (1985). *Ibid.* 51 : 277-284.

Table 1. AK-toxin production in spore-germinated fluids of pathogenic and nonpathogenic isolates of *A. alternata* Japanese pear pathotype

Population	Number of isolates						
	AK-toxin production ^{a)}						
	<2 ⁰	2 ⁰	2 ¹	2 ²	2 ³	2 ⁴	2 ⁵
Nonpathogenic isolates	36	0	0	0	0	0	0
Pathogenic isolates	0	12	10	12	5	2	1

a) AK-toxin production was represented by the dilution end point for toxicity of spore-germinated fluids harvested after 24 hr of incubation at 25 C.

ly lost the ability to produce the HST, hence became saprophytes unable to colonize on host plant leaves.

和 文 摘 要

柘植尚志・林 律器・西村正陽：*Alternaria alternata* 群病原菌における宿主特異的毒素生成の制御 (3). ナシ黒斑病菌野生株における病原性の不安定性

二十世紀ナシ葉上の黒斑病病斑から、多数の単孢子分離菌株を得、それらの病原性の不安定性について、分生孢子発芽時の AK 毒素生成能にも注目しながら検討した。10個の病斑より分離した197菌株のうち、28菌株はすでに分離直後に病原性を失っていた。さらに、一病斑当り10菌株ずつ選ばれた100菌株を継代培養したところ、病原性失活菌株が徐々に出現した。病斑ごとに精査してみると、新植二十世紀ナシ園の病斑からは、概して、病原性の不安定な菌株が多く得られ、永年栽培の持続しているナシ園の病斑からは、病原性の比較的安定な菌株が得られた。病原性失活菌株は、例外なく、AK 毒素生成能を喪失していたが、培養菌糸や分生孢子子の形態に何ら変化も認められなかった。

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