71. Determinant for Pathogenicity without Apparent Phytotoxicity in Plant Diseases

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“What’s the pathogenicity?” is the most fundamental question in the field of parasitology, but the informations to answer this question is insufficient especially in plant diseases.

Plant pathogenic microorganisms are assumed to be evolved from saprophytic ones through acquisition of parasitic properties by adaptation, in order to derive the food materials from plants. According to the grade and type of adaptation, there are varieties of microorganisms whose parasitism is, in varying degrees, from facultative to obligatory. For example, facultative parasites which usually live saprophytically but can parasitize plants under suitable conditions are likely to be the most primitive type of pathogens whose parasitic adaptation is the lowest grade. *Erwinia carotovora*, a pathogenic bacterium of vegetables established infection through wounds in wide range of plant species. This bacterium usually attacks the storage organs in a semidormant or dormant condition, such as carrot roots, potato tubers, onion bulbs, fleshy petioles of celery and so on. The pathogenicity of this bacterium was attributed to the high productivity of pectolytic enzymes. *E. carotovora* produced 7–8 times as much pectic acid trans-eliminase compared with a pure saprophytic bacterium, *Pseudomonas fluorescens* when cultured on pectin medium supplemented with potato tuber disks.

On the contrary, the uppermost level of parasitic adaptation can be seen in the race-cultivar specificity of some diseases in cultivated plants, where a race infects and causes disease in only a definite cultivar of a plant species but not another cultivar of the same species. The other race has its own cultivar as the host. In such host-parasite combinations, enzymes and/or toxins of deleterious activity to varieties of plant cells can hardly be the determinant of pathogenicity of primary importance. In these diseases, there must be highly specific mechanism of interactions between host and parasite.

The relationship between host-specific toxins of pathogen's origin and the toxin receptors in the compatible hosts is the best explanation of host-parasite specificity in some plant diseases. However, only 13
species of pathogenic fungi were found to produce host-specific toxin at present, and moreover, there is no possibility that such a drastic toxin might concern the host-parasite interactions in the obligate parasitic diseases. It seems to be probable that some determinants of pathogenicity without drastic phytotoxicity might concern the host-parasite interaction of obligate parasitism.

The phenomena of induced susceptibility or accessibility by double inoculation technique in obligate parasitic diseases provided a concept that the most important task of pathogenic organisms in diseases of distinct host-parasite specificity might be the suppression of the defense reaction in their own hosts.

In potato late blight disease, a substance which prevents hypersensitive reaction in potato tuber or petiole tissue by inoculation with incompatible race of Phytophthora infestans was isolated from zoospores and mycelia of the compatible race and found to be a glucan having 1,3- and 1,6-glucosidic linkage in its molecule. Unfortunately, this substance did not affect the infectivity and the growth of intracellular hyphae of incompatible races. A suppressor of host-resistance reaction was found to be produced by a pea pathogen, Mycosphaerella pinodes in our laboratory as follows. Namely, when the pea leaf or stem was inoculated with incompatible or some nonpathogenic fungi of pea, pisatin, a post-infectional antifungal substance accumulated in the inoculated sites within 10 hr after inoculation. However, by inoculation with virulent pathogen, the accumulation of pisatin at this stage was not detectable, and became detectable after the infection was established, i.e., 24 hr after inoculation. Pea pathogens such as Erysiphe pisi and M. pinodes were not inhibited their spore germination or germ-tube elongation by 200-500 ppm of pisatin, but the penetration ability of these fungi into pea leaves or cellophane sheets was inhibited almost completely at a concentration of 50 ppm. These facts suggested that the pea pathogen must have the mechanism to suppress the accumulation of pisatin at an early stage of infection in order to avoid its inhibitory activity to infection (hyphal penetration).

As the results of experiments carried out following the above ideas, we demonstrated that a pea pathogen, M. pinodes releases suppressors and elicitors of pisatin biosynthesis into the germinating fluids of spores. The elicitor was found to be a high-molecular-weight polysaccharide and the activity was inactivated by the substance of low-molecular-weight concomitantly present in the germinating fluids. Purification of suppressor from low-molecular-weight fraction by gel filtration and thin layer chromatography yielded two ninhydrin
positive, active substances, and tentatively named as F2 and F5. F5 was more active than F2, and suppressed pisatin accumulation completely at a concentration of 50 ppm (as protein by Lowry method) by subsequent inoculation with Stemphylium saricinaeforme or Erysiphe graminis hordei. Further, 50 ppm of F5 allowed nonpathogen of pea, such as Cochliobolus miyabeanus, Mycosphaerella melonis, Alternaria alternata 15B (avirulent isolate of Japanese pear pathotype), and S. sarcinaeforme to infect on pea leaves. Among these, A. alternata 15B and S. sarcinaeforme colonized and formed conidia on F5-treated pea leaves 14 days after inoculation. As far as tested, M. pinodes, the F5 producer could infect 5 species of leguminous plants, Pisum sativum, Trifolium pratense, Millettia japonica, Lespedeza buergeri, and Medicago sativa. A. alternata 15B could not infect on any of the leguminous plants tested, but could infect and formed intracellular hyphae on 5 species of plants to which M. pinodes was pathogenic in the concomitant presence of 50 ppm of F5. In other words, the host specificity of M. pinodes coincided with the specificity of the biological activity of F5. Purified F5 is composed of a peptide as the active centre, and the amino acid composition was glycine, aspartic acid and serine at the ratio of 1:1:2. F5 did not cause any visible injury to pea leaf cells at the microscopic level, therefore it can hardly be a “host-specific toxin”.

These facts suggest that suppressors especially F5 produced by the pea pathogen, M. pinodes might be a determinant of pathogenicity by breaking down the general defense mechanism of some plants without apparent phytotoxicity, and could parasitize on these plants.

Gäumann described that plant pathogens must possess the capacity to infect its host, to inhabit in it, to overcome its resistance, and to reproduce itself in or on it. These capacities are called aggressiveness. In addition, the ability of pathogen to evoke disease is called pathogenicity or virulence. Among these, the following three capacities might be the fundamental properties which plant pathogens acquired by parasitic adaptation: Abilities to enter into plants, to overcome host resistance and to evoke disease. On the 1st ability, primitive pathogens enter through wounds, but highly specialized pathogens have their proper ways, stomatal or cuticular infection. Pathogenic fungi that penetrate through unbroken plant surface were known to penetrate artificial membranes such as cellophane and collodion-paraffin-wax membrane, and these properties are the 1st step of the parasitic adaptation of pathogens.

As to the ability to overcome the resistance of host, our suppressor, F5 might be the best example and probably suppressors of this type (without phytotoxicity) play a key role in the pathogenicity
of many obligate parasites. In fact, a suppressor of pisatin biosynthesis was found in germinating conidia of *Erysiphe pisi*.\(^{18}\) The specificity of suppressor which suppresses the function of resistant gene of higher plants might determine the host-parasite specificity. Recently, evidence was provided that AK-toxin, a host-specific toxin produced by *A. alternata* Japanese pear pathotype acts as the suppressor of resistant reaction on its own host at an early step of infection.\(^{6}\)

The last properties which evoke diseases are mainly concerned with toxin or drastic enzyme activities produced by pathogen or by the result of host-parasite interactions. There seems to be no possibility that such a drastic toxin of pathogen's origin may concern with the obligate parasitism.

From these viewpoints, host-specific toxins seem to have two of the three above mentioned-important abilities of pathogenicity, the ability to suppress the resistant reaction and to evoke disease.

Though we demonstrated the importance of a suppressor without apparent phytotoxicity in the *Mycosphaerella* blight disease of pea, another substance which evoke cell death may be involved in the pathogenesis of this disease because the pathogenic fungus, *M. pinodes* is not an obligate parasite.

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References