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Mycological Characteristics of *Fusarium solani* f. sp. *pisi* Isolated from Pea, Ginseng and Soybean in Korea

Yong Sung Jung, Young Tae Kim, Sung Joon Yoo and Hong Gi Kim* 김홍기

Department of Agricultural Biology, College of Agriculture, Chungnam National University, Taejon 305-764, Korea

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Fungal isolates belong to *Fusarium solani* (perfect stage : *Nectria haematococca*) were isolated from pea, ginseng roots and soybean during 1995 and 1996 in Korea. Thirty-five isolates were identified as *F. solani* f. sp. *pisi* based on the morphological characteristics and their pathogenicity. Microconidia formed on the long conidiophore were ovoid or oblong sizing 5~14×2.5~5.0 μm. Macroconidia were formed on the multibranched conidiophores on carnation leaf agar media with 4.8~5.3×32.0~40.7 μm in size. They belonged to β-type since their 3-septate macroconidia were smaller than 5 μm in width. Chlamydospores were smooth- or rough- walled and formed in terminal or lateral branches of hyphae, intercalary, or in chains. Most isolates were highly virulent to pea seedlings, producing dark brown or rot lesions. This is the first report of *F. solani* f. sp. *pisi* being pathogenic to pea in Korea.

Keywords : *Fusarium solani* f. sp. *pisi*, β-type, identification, pea.

Fusarium solani (Mart.) Appel Wollenw. emend. Snyder Hansen includes a number of important pathogens to crops such as peas, Korean ginseng and cucurbits (Abney and Richards, 1993; Killebrew et al., 1988; Nelson et al., 1983). They are also able to develop a disease on at least one animal species and inhabit exist as soil- or plant- inhabiting saprophytes (VanEtten and Kistler, 1988). *Fusarium solani* is identified based mainly on the morphology of both the asexual spores and the structures bearing the spores following Booth's division. At the subspecies level, formae speciales is a frequently used trinomial that signifies mainly the pathogenicity.

Nectria haematococca Berk. Br. (Syn. *Hypomyces solani*) is known as the perfect stage of *F. solani* (Hanlin 1971; Nelson et al., 1983). Both homothallic and heterothallic isolates of *N. haematococca* are known and seven distinct mating populations (MP) which can not interbreed (Matuo and Snyder, 1973; VanEtten, 1978; VanEtten and Kistler, 1988) are known among the heterothallic isolates. A conventional genetic analysis has been conducted with one homothallic strain, MPI (pathogenic to cucurbit), and MPVI (pathogenic to pea). Most of virulent strains studied so far belong to heterothallic group but homothallic strains has rarely shown virulence. MPI and MPVI have been studied for the pathogenicity of *F. solani*, and could be easily distinguished each other by the resistance to toxin, chromosome number, mitochondrial genome size, and RFLP pattern of rDNA (VanEtten and Kistler, 1988).

Several works (Denny and VanEtten, 1983; Maloney and VanEtten, 1994; Miao et al., 1991; VanEtten et al., 1989) have shown that *F. solani* f. sp. *pisi* (perfect stage : *N. haematococca* MP VI) is amenable to conventional genetic analysis, suggesting that this fungus could serve as a useful organism for the genetic and physiological studies on the molecular basis of pathogenicity. Furthermore, the genetic variation in pathogenicity has already been identified among members of MP VI. This variation includes naturally occurring differences in the ability to detoxify the pea phytoalexin pisatin by a cytochrome P-450 class enzyme called pisatin demethylase (Van Etten et al., 1989).

The purpose of this study was to isolate and identify *F. solani* and to determine formae speciales based on their pathogenicity in various hosts to facilitate further physiological and molecular genetic studies on this organism.

Materials and Methods

Isolation. *Fusarium solani* isolates were obtained from the diseased peas, ginseng roots and soybeans from various locations in Korea (Table 1). The stem and root portion of plants bearing lesions characteristic to *F. solani* infections were washed in distilled water. The lesions were surface-sterilized with 1 % sodium hypochlorite for 3 min and dried on filter paper in petri dishes and then layered onto peptone pentachloronitrobenzene agar plate. After incubation for 24~48 hr at 25°C, conidia were collected from conidiophores emerging from the infected tissue and transferred to the water agar (WA). Mycelial tip or single spore was isolated from the colonies bearing the microconidiophores characteristic to *F. solani*.

Identification. Morphology of the fungi was examined following procedures described by Matuo and Snyder (1973) and Synder

*Corresponding author.
Phone) +82-42-821-5768, Fax) +82-42-823-8679
E-mail) hgkim@hanbat.chungnam.ac.kr

Table 1. Isolates of *Fusarium* spp. used in this study originated from Korea and U.S.A.

Isolate	Geographic origin	Host
T-2[a]	U.S.A.	*Pisum sativum*
44-100[a]	U.S.A.	*Pisum sativum*
FSP401403	Seosan, Chungnam	*Pisum sativum*
FSP404413	Yuseong, Taejon	*Pisum sativum*
FSPA10112	Pyoungtaek Kyongki	*Panax ginseng*
FSPA201204	Hyengsung, Kangwon	*Panax ginseng*
FSPA301304	Uisung, Kangwon	*Panax ginseng*
FSPA401404	Nonsan Chungnam	*Panax ginseng*
FSPA701703	Iri, Chunbuk	*Panax ginseng*
FSG101	Yeuju Kyongki	*Glycine max*
FSG301303	Boun, Chungbuk	*Glycine max*
FSG304	Umsung, Chungbuk	*Glycine max*
FSG401402	Chungyang, Chungnam	*Glycine max*
FSG403407	Yuseong, Taejon	*Glycine max*
FSG501505	Andong, Kyungbuk	*Glycine max*
FSG506508	Chongsong, Kyungbuk	*Glycine max*
FSG509611	Euisung, Kyungbuk	*Glycine max*
FSG601602	Hapchun, Kyungnam	*Glycine max*
FSG603609	Changryeng, Kyungnam	*Glycine max*

[a]These *Nectria haematococca* MPVI strains were kindly provided by Dr. H.C. Kistler, University of Florida, U.S.A.

Hansen (1953). The isolates were cultured on potato dextrose agar and carnation leaf agar (CLA). Morphological characteristics such as elongated conidiophores and monophialides forming microconidia on PDA were examined under a light microscope at ×100 magnification. The size, shape of macroconidia and multibranched conidiophore of the isolates cultured on CLA at 28°C, were examined under a light microscope at ×400 3~7 days after incubation. The isolates were assigned with a code number and stored in two ways. First, all isolates were transferred to PDA slant, kept at 27°C for 1 week and then stored in the dark room at 4°C. Second, the margins of colonies growing on PDA were cut with a cork borer (7 mm in dia.) and the disk were stored in distilled water in a 1.5 ml-microtube at a room temperature.

Inoculation and formae speciales determination. Test tubes containing vermiculite and 20 ml of Haglund's solution were prepared (Haglund, 1989; VanEtten, 1978). Pea seeds were surface-sterilized using 30% sodium hypochlorite for 3 min and then thoroughly rinsed with distilled water to remove excess sodium hypochlorite. Seeds were planted in the test tube 1 cm deep. Seedlings were grown for 2~3 week at 28~30°C under light or until peas were in the 4~5 node stage of development. The fungus grown on M-100 minimal medium (MM) for 2~3 days were cut into disk plugs (7 mm in dia.) with a cork borer. A wound was made on stem with a dissecting needle. The inoculum discs were attached to the wound. Five test tubes per isolate were prepared in this way. The plant tissues were supplied with sufficient Haglund's solution for the test period. After 2 weeks, the length at the inoculation site was measured. Avirulent isolate, 44-100, was used as a negative control and remained disease free on pea. A highly virulent isolate, T-2 obtained from U.S.A, was also used as a positive control.

Results

Identification. Eighty isolates of *F. solani* were obtained from diseased peas, ginseng roots and soybeans in Korea. All the isolates produced abundant microconidia on lateral conidiophores (Fig. 1-A). Conidia were ovoid or oblong measuring 51~4×2.0~5.0 μm. Monophialides and long conidiophores of the isolates were distinguished from short conidiophores of *F. oxysporum* isolates.

All isolates cultured on CLA produced macroconidia after 7 days on the multibranched conidiophores (Fig. 1-B). They had 1~4 septate macroconidia, predominantly 3-septate. Macroconidia had more rounded and thickened foot cells than those of *F. oxysporum*. Sizes of macroconidia were averaged 4.8~5.3×32.0~0.7 μm. Predominant 3-septate macroconidia were smaller than 5 μm in width (Fig. 1-C). Characteristic to the β-type belong to *F. solani* f. sp. *pisi* was that chlamydospores were formed readly on the MM or PDA with smooth- or rough-wall. Chlamydospores were also formed on terminally lateral branches or intercalary and occasionally in chains (Fig. 1-D).

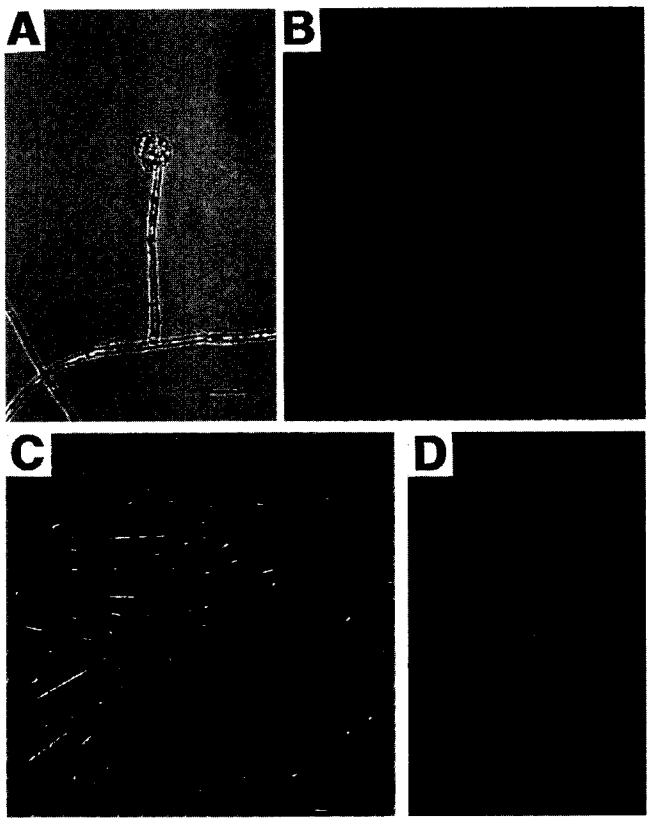

Fig. 1. The morphological characteristics of *Fusarium solani* f. sp. *pisi*. A; Microconidia with a long conidiophore. B; Macroconidia on multibranched conidiophores. C; 3-septated macroconidia of β-type (smaller than 5 μm in width). D; Chlamydospores formed on hyphae terminal or intercalary. The scale bar represents 10 μm.

Table 2. Virulence of *Fusarium solani* isolates to pea seedlings

Isolate	Pathogenicity	Isolate	Pathogenicity
T-2	++++[a]	FSPA110	++++
44-100	–	FSPA111	++
FSP401	++++	FSPA112	+
FSP402	++++	FSPA113	–
FSP403	+++	FSPA114	+
FSP404	++	FSPA116	–
FSP405	++	FSPA117	–
FSP406	++++	FSPA118	–
FSP407	++++	FSPA119	+
FSP408	++++	FSPA120	++
FSP409	++++	FSPA121	+++
FSP410	++	FSG101	++++
FSP411	+++	FSG301	+
FSP412	+	FSG302	++
FSP413	++	FSG304	++++
FSPA101	+++	FSG403	++++
FSPA102	++	FSG405	–
FSPA103	++++	FSG501	–
FSPA104	++++	FSG502	++++
FSPA105	+++	FSG508	–
FSPA106	++	FSG509	++
FSPA107	+++	FSG606	++++

[a] ++ and + : weak virulent, +++ : moderately virulent, ++++ : highly virulent, – : avirulent

Symptom and pathogenicity. Pea plants infected by artificial inoculation showed initially round or irregular light brown lesion and progressed into dark brown lesions on stems. Finally leaves and stems infected were died. One to two weeks after inoculation with the virulent isolates, black lesions appeared at the inoculation site, were the plants inoculated with avilulent isolates remained healthy. The lesions by highly virulent isolates were generally dark brown and stems at the inoculation site were completely rotten. Table 2 shows the results of the pathogenicity test of the isolates on peas. About half of the isolates from peas, ginseng roots and soybeans showed a high virulence to pea. Thus these isolates were identified as *F. solani* f. sp. *pisi* bassed on the pathogenicity and morphological traits.

Discussion

Fusarium solani f. sp. *pisi* found in a variety of habitats including pea, ginseng, mulberry or chickpea. However, *F. solani* f. sp. *pisi* was not found in soybean (Matuo and Snyder, 1973). The pathogenic fungus to soybean was known as just *F. solani* (Abney and Richards, 1993; Killebrew et al., 1988). This fungus was used as a very useful research organism for genetic studies on the molecular basis of pathogenicity (Maloney and VanEtten, 1994; Miao et al., 1991; VanEtten et al., 1989). Thus it will be useful to get some basic informations including isolation and classification into formae speciales of various isolates. For this purpose we collected eighty isolates from peas, ginseng roots and soybeans during 1995 and 1996 in Korea. Most morphological characteristics of the isolates were matched with *F. solani* f. sp. *pisi* described by different authors (Matuo and Snyder, 1973; Synder and Hansen, 1953; VanEtten, 1978). Although morphology of microconidia produced on the conidiophore is a basic criterion to classify *F. solani*, it is more important to examine the short multibranched conidiophores and morphological type of macroconidia (Matuo and Snyder, 1973). Nine formae speciales and two races of *F. solani* have been reported in other countries (Abney and Richards1, 1993; Killebrew et al., 1988; VanEtten, 1978). They were sorted into four types (α, β, γ, δ) by the morphological characteristics of macroconidia which were formed on sporodochia. *Fusarium solani* had a variety of formae speciales and sorted into four types characterized by septate number of macroconidia described by Matuo and Snyder (1973). Macroconidia of *F. solani* f. sp *pisi* had known as β-type that had 1~4 septate macroconidia, predominant 3-septate macroconidia (less than 5 μm in width) and 4.5~6×35~55 μm or 58~45×100 μm in size. Furthermore, the γ-type *F. solani* f. sp. *radicicola* was very similar to the β-type on macroconidia; they could be distinguished by the width of 3-septate macroconidia; one had 5.5 μm or more in width of 3-septate macroconidia, the other had less than 5 μm in width of them. The α-type, composed of f. sp. *cucurbitae* race 1 and some isolates of race 2, f. sp. *batas*, f. sp. *mori*, f. sp. *xanthoxyli*, f. sp. *robiniae* and an isolate of f. sp. *eumartii* is characterized by the predominant 5 or more septate macroconidia. The β-type, included f. sp. *pisi*, f. sp. *cucurbitae* race 2 and some isolates of f. sp. *eumartii*, have predominant 3-septate macroconidia (smaller than 5 μm in width). The γ-type, f. sp. *radicicola*, is characterized by the predominant 3-septate macroconidia larger than 5.5 μm in width. The δ-type, f. sp. *phaseoli*, has predominant 4-septate macroconidia. There was no significant difference between the morphology of microconidia and *Hypomyces* stage among these formae speciales and races (Matuo and Snyder, 1973). All isolates used in this study formed microconidia on long conidiophore differing from those of *F. oxysporum* on PDA. Macroconidia of the isolates were a β-type which had 1~4 septate, predominantly 3-septate macroconidia (smalller than 5 μm in width) and they showed short multibranched conidiophores on CLA.

Traditionally, the formae speciales classification concerns pathogenicity rather than morphological characteristics. In the pathogenicity test to pea, about half of the isolates from peas, ginseng and soybean showed high virulence to pea seedlings, but a few ginseng roots and soybean

isolates showed weak or no virulence to pea. There were, however, generally no remarkable virulence differences to pea regardless of sources. In addition to pathogenicity, the crossing test for mating and sexual behavior may be useful tools for identification of formae speciales and races (Hanlin, 1971; Matuo and Snyder, 1973; VanEtten, 1978). Based on the results of morphological characteristics and pathogenicity test, the isolates collected from pea, soybean and Korean ginseng could be classified as *F. solani* f. sp. *pisi*. This is the first report of the occurrence and pathogenicity of *F. solani* f. sp. *pisi* to pea in Korea.

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