***Fusarium* species: Mycotoxin production, and plant and murine pathogenicity**

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Summary

Fusarium species are common filamentous fungi, and are distributed worldwide in crop fields. Some of the species are known to produce mycotoxins such as trichothecenes, zearalenone, and fumonisins, which are responsible for mycotoxicoses in humans and animals. Since 1980, I have been studying the chemical characteristics of selected *Fusarium* species, along with plant and murine pathogenicity caused by *Fusarium crookwellense* and *Fusarium solani*, respectively. In this review, I outline my experimental *Fusarium* studies, including some experiences during those studies.

Key words: *Fusarium*, *Gibberella*, mycotoxins, chemotype, pathogenicity

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Introduction

Fusarium species are a widespread cosmopolitan group of filamentous fungi that distribute commonly in soil in agricultural fields. Some of these species are major causal agents of several crop diseases such as 'head blight (scab)' ('Akakabi-byo in Japanese') in wheat and barley, and 'ear rot' in maize in infected kernels¹⁾. In Japan, *F. graminearum* and its teleomorph *Gibberella zeae* are major causative agents responsible for sporadic epidemics of scab diseases in wheat, barley, and rice. The natural occurrence of Akakabi-byo disease has resulted in national production yield losses of wheat and barley grains²⁾. According to the field study of Nishikado³⁾, scab diseases have been frequently documented in spring and autumn in wheat fields. Due to the moderate temperatures and high humidity in those seasons, the climate is suitable for conidia or ascospore dispersal of *F. graminearum* or *G. zeae* in crop fields. The prevailing distribution of those species results in a high incidence of scab diseases in wheat and barley fields.

Fusarium species are known to produce mycotoxins such as trichothecenes, zearalenone, and fumonisins. Cereal grains contaminated with trichothecenes are associated with outbreaks of human mycotoxicosis characterized by anorexia, nausea, vomiting, headache, abdominal pain, diarrhea, chills, and convulsions⁴⁾. Trichothecenes are a major mycotoxin produced by some toxigenic *Fusarium* species including *F. graminearum*, *F. sambucinum*, *F. sporotrichioides*, *F. equiseti*, *F. crookwellense* (= *F. cerealis*), *F. culmorum*, *F. poae*, and *F. acuminatum*. Based on chemical features of the trichothecene nucleus, the trichothecenes produced by *Fusarium* species belong to two categories⁴⁾: type A-trichothecenes characterized by a functional group other than a keton at C-8, and type B-trichothecenes with only the carbonyl at C-8. The type A-trichothecenes are T-2 toxin (T-2) and HT-2 toxin, mainly produced by *F. sporotrichioides*, *F. acuminatum* and *F. poae*, and diacetoxyscirpenol (DAS) produced by *F. poae*, *F. equiseti*, *F. sambucinum* and *F. sporotri-*

chiioides. The type B trichothecenes are deoxynivalenol (DON), and 3-acetyl DON (3-ADON), 15-acetyl DON (15-ADON), and 3, 15-diacetyl DON produced by *F. graminearum* and *F. culmorum*, and nivalenol (NIV), and fusarenon-X (FX) or 4-acetyl NIV (4-ANIV), and 4, 15-diacetyl NIV produced by *F. graminearum*, *F. crookwellense* and *F. poae*. Natural occurrence of the trichothecenes has been observed worldwide in wheat, barley, corn, soybeans and related products⁵⁾.

Among the type B trichothecenes, NIV, FX, and DON were found for the first time by Japanese mycotoxin researchers. In 1968, Tatsuno and co-workers⁶⁾ reported NIV as a new toxic metabolite, which was isolated from rice cultures of *Fusarium* strain Fn-2B. The strain was previously called *Fusarium nivale*, but is now classified as *F. kyushuense*⁷⁾. The following year, Ueno and co-workers⁸⁾ found FX, a 4-acetyl derivative of NIV, in liquid cultures of the Fn-2B strain. In 1973, Yoshizawa and Morooka⁹⁾ reported DON and its acetyl derivatives from cultures of *Fusarium roseum* (= *F. graminearum*). A few months later, vomitoxin was reported as a toxic metabolite of *F. graminearum* by Vesonder, et al.¹⁰⁾ in the United States. DON and vomitoxin had exactly the same chemical structure, and the chemical name DON had priority over that of vomitoxin due to the date of publication. As a result, the isolation of NIV, FX, and DON was done by Japanese scientific researchers. Currently, these naturally occurring trichothecenes are of great concern as a worldwide problem in wheat and barley cultivated in agricultural fields.

On July 2011, a change in the nomenclature for fungi was made in the 18th International Botanical Congress held in Melbourne¹¹⁾. The Congress decided to use 'One fungus one name'. However, the meetings will continue to discuss which genus or species name of the anamorph-teleomorph should be used until 2018. In this review, I use two names for one fungus based on the anamorph and teleomorph, which are described in the monograph of the genus *Fusarium*.

Chemotypes of *Gibberella zeae*, and the teleomorph state of *Fusarium graminearum*

In my graduate course at the Faculty of Pharmaceutical Sciences, Science University of Tokyo, I studied the geographic distribution of *Gibberella zeae* and the production of trichothecenes. *G. zeae* is the name of the teleomorph state of *F. graminearum*, and it produces perithecia, which are ovoid with a very rough tuberculate wall and are dark purple or black. The perithecia of *G. zeae* are found on a wide range of gramineous hosts, especially on rice stubble and gramineous grass debris in crop fields in Japan³⁾. Before starting my study, the natural occurrence of trichothecenes in wheat and barley cultivated in Japan had been reported by several Japanese researchers^{12,13)}. The data suggested that the presence of DON and NIV may show geographical differences, possibly associated with the distribution of toxigenic *Fusarium* species¹²⁾. Based on these previous studies, I collected the perithecia of *G. zeae* in 3 different regions under the direction of Dr. Masakatsu Ichinoé, who worked for the National Institute of Hygienic Sciences at that time.

By using single spore isolation, a single ascospore was randomly chosen from a mass of ascospores discharged from a mature perithecium. Seven ascospores per perithecium were obtained from the samples harvested. Among them, 113 isolates of *G. zeae* were tested for the production of trichothecenes. As a result, all isolates of *G. zeae* produced trichothecenes, and they were divided into two groups: DON-producing isolates and NIV-producing ones. As each DON and NIV is an independent end-product in the trichothecene biosynthetic pathway (Fig. 1), I proposed to introduce the concept of 'chemotype' for the two groups of *G. zeae*, referring to the nomenclature rule for fungi. Therefore, I used '*G. zeae* DON-chemotype' and '*G. zeae*

Fig. 2 Two chemotypes of *Gibberella zeae*

question arose because random isolation of ascospores discharged from a perithecium was used to determine the chemotypes of *G. zeae* in a previous study¹⁴. To answer this question, I separated each of eight ascospores from a single ascus by dissection. Then, each ascospore was examined for the production of trichothecenes. As shown in Table 1, each of eight ascospores was characterized with the same chemotype¹⁶.

In a previous study¹⁶, some ascospores from Miyagi Prefecture produced both DON and NIV, but their toxin levels were not equal. This suggested that crossing of *G. zeae* with two different chemotypes may occur in the field. Furthermore, *G. zeae* from Hirosaki in Aomori Prefecture produced only DON, but its precursors were two different acetyl derivatives, 3-ADON and 15-ADON. As shown in Table 1, each of the TK-1 and TH-4 isolates produced different types of acetyl derivatives; 3-ADON, 15-ADON, or both. However, all of these isolates produced only 15-ADON when cultured at 25 °C for 14 days followed by culture at 4 °C for 14 days (Table 2). The reason for the change to produce only 15-ADON was unclear at that time. Currently, the genetic basis for the difference in 3-ADON and 15-ADON has been identified¹⁷, and further studies may solve the mystery of 15-ADON only production by low temperature treatment.

In 1982 during my graduate course, I studied the isolation and identification of *Penicillium* species obtained from several Indian rice grains under the guidance of Dr. Hiroshi Tsunoda. He was a famous scientist in Japanese mycotoxin research because he found for the first time NIV-producing *Fusarium* species from rice straws in the Kyushu region. While working with him, I heard his hypothesis on Akakabi-byo disease occurring in the crop fields of Japan. His theory was that spores of toxigenic *Fusarium* species were brought to Japan from China by seasonal a phenomenon known as “Kosa” in Japanese.

Actually, wind dispersion of *Fusarium moniliforme* conidia as far as 400 km has been reported in the United States¹. However, I did not understand his explanation at that time. Later, I recalled his ‘Kosa’ hypothesis when I found some perithecia of *G. zeae* formed on the debris of a graminaceous grass near the top of a mountain in the Kyushu region. I tried taking spores of the *Fusarium* species on a flight, but I did not get the chance to clarify his hypothesis. Now, genetic approaches are a practical and useful tool for clarifying the geographical differences in toxigenic *Fusarium* strains derived from Japan or China. I hope that young

Table 1. Production of 8-ketotrichothecenes by *Gibberella zeae* strains

Group and strain	8-keto-trichothecene	Production (µg/g) of 8-ketotrichothecene by individual ascospores from an ascus							
		1	2	3	4	5	6	7	8
DON group									
TK-1	NIV	ND	ND	ND	ND	ND	ND	ND	ND
	4-AcNIV	ND	ND	ND	ND	ND	ND	ND	ND
	DON	53.83	28.59	34.81	43.37	36.70	33.07	29.88	24.55
	3-AcDON	3.54	1.57	2.41	2.00	2.94	2.34	1.46	1.53
	15-AcDON	ND	1.13	ND	ND	ND	2.72	ND	ND
TK-2	NIV	ND	ND	ND	ND	ND	ND	ND	ND
	4-AcNIV	ND	ND	ND	ND	ND	ND	ND	ND
	DON	3.69	4.06	3.08	5.17	4.04	2.10	3.31	2.65
	3-AcDON	ND	ND	ND	ND	ND	ND	ND	ND
	15-AcDON	1.75	1.37	1.57	1.76	1.27	1.03	1.49	0.69
TH-4	NIV	ND	ND	ND	ND	ND	ND	ND	ND
	4-AcNIV	ND	ND	ND	ND	ND	ND	ND	ND
	DON	22.36	26.43	18.73	17.98	48.27	12.20	32.98	19.38
	3-AcDON	ND	1.69	1.80	ND	2.16	ND	2.07	1.86
	15-AcDON	4.82	1.07	2.91	9.84	ND	4.18	1.25	2.12
TH-5	NIV	ND	ND	ND	ND	ND	ND	ND	ND
	4-AcNIV	ND	ND	ND	ND	ND	ND	ND	ND
	DON	186.52	203.64	76.66	160.52	272.18	127.46	366.97	146.90
	3-AcDON	ND	ND	ND	ND	ND	ND	ND	ND
	15-AcDON	11.84	12.66	5.23	8.96	26.68	11.30	64.17	9.45
NIV group									
TY-1	NIV	15.52	4.77	102.43	13.06	11.54	6.41	12.86	0.97
	4-AcNIV	5.78	2.13	15.70	5.27	5.43	2.41	5.56	ND
	DON	0.32	0.09	0.74	0.45	0.21	0.09	0.43	0.01
	3-AcDON	ND	ND	ND	ND	ND	ND	ND	ND
	15-AcDON	ND	ND	ND	ND	ND	ND	ND	ND
MM-1	NIV	0.75	0.66	0.75	0.24	0.18	0.06	0.87	0.60
	4-AcNIV	0.11	0.10	0.23	0.05	0.03	0.04	0.05	0.26
	DON	0.02	0.01	ND	ND	ND	ND	ND	ND
	3-AcDON	ND	ND	ND	ND	ND	ND	ND	ND
	15-AcDON	ND	ND	ND	ND	ND	ND	ND	ND
SC-1	NIV	0.57	0.20	0.69	0.69	0.34	1.53	0.66	0.62
	4-AcNIV	0.16	0.01	0.11	0.10	0.05	0.30	0.25	0.04
	DON	ND	ND	0.02	ND	0.01	ND	ND	ND
	3-AcDON	ND	ND	ND	ND	ND	ND	ND	ND
	15-AcDON	ND	ND	ND	ND	ND	ND	ND	ND

scientists will prove Tsunoda's hypothesis; toxigenic *F. graminearum* are blown by winds to Japan together with Chinese soil ('Kosa').

Table 2. Production of 8-ketotrichothecenes with TK-1 and TH-4 strains after incubation at 25 °C for 14 days followed by 4 °C for 14 days

Group and strain	8-keto-trichothecene	Production (µg/g) of 8-ketotrichothecene by individual ascospores from an ascus							
		1	2	3	4	5	6	7	8
TK-1	NIV	ND	ND	ND	ND	ND	ND	ND	ND
	4-AcNIV	ND	ND	ND	ND	ND	ND	ND	ND
	DON	27.79	26.90	40.75	42.86	10.77	38.31	12.57	30.82
	3-AcDON	ND	ND	ND	ND	ND	ND	ND	ND
	15-AcDON	9.37	2.76	5.35	4.06	3.72	5.92	9.27	5.44
TH-4	NIV	ND	ND	ND	ND	ND	ND	ND	ND
	4-AcNIV	ND	ND	ND	ND	ND	ND	ND	ND
	DON	13.16	13.68	7.40	19.95	10.62	23.45	21.51	9.50
	3-AcDON	ND	ND	ND	ND	ND	ND	ND	ND
	15-AcDON	7.82	7.36	2.98	4.87	5.89	4.85	3.20	5.44

Natural occurrence of nivalenol in wheat and barley in Hokkaido

The contamination of wheat and barley with DON and/or NIV has been observed frequently in Japan. Geographic differences in DON and NIV have been investigated in grain samples of wheat and barley harvested in several areas of Japan^{12,13}. In Kyushu and Shikoku, DON is predominant over NIV, and NIV is predominant over DON from Chugoku to Tohoku. In the northernmost island, Hokkaido, DON is dominant in the wheat fields. In previous studies, the isolates of *F. graminearum* derived from wheat grains in Hokkaido were only DON producers¹⁸. NIV contamination of wheat grains cultivated in Hokkaido was observed, but the fungi responsible for the natural occurrence of NIV were not identified¹⁹. A mycological survey in Hokkaido demonstrated the presence of NIV-producing *Fusarium poae* in scabby wheat grains harvested in the Tokachi district¹⁸.

Based on previous studies, I tried to find fresh toxigenic *Fusarium* species responsible for NIV production elsewhere in Hokkaido. On July 1991, I went to 19 wheat fields in eight locations in six districts of Hokkaido, Ebetsu, Shimidzu, Tokoro, Nayoro, Hippu, Furano, Kyogoku, and Hakodate, with the special assistances of agricultural agents who graduated from the Rakuno Gakuen University (lab students of Prof. Yukio Matsui). As a result of the mycological survey, *F. poae* and *Fusarium crookwellense* were found to be NIV producers responsible for the natural occurrence of wheat grains with NIV in Hokkaido²⁰. Especially, four isolates of domestic *F. crookwellense* were obtained for the first time from scabby wheat grains sampled in the Kyogoku district. Unfortunately, there are no reports on the natural distribution of *F. crookwellense* in other regions of Japan, except Hokkaido.

The isolates of *F. crookwellense* obtained from scabby wheat were tested for plant pathogenicity against wheat and barley plants with the kind help of Dr. Hatsuo Saitoh, who worked for the Tohoku National Agricultural Experiment Station at that time. All isolates tested were found to be virulent in wheat and barley plants within 15 days after fungal spore inoculation. Symptoms were mainly observed on the heads of test plants and were identified as scab disease. The pathogenic potential of *F. crookwellense* isolates was almost the same as that of a wheat pathogenic *F. graminearum* strain, which was used as a positive reference²¹. In

conclusion, it was demonstrated that the contamination of wheat and barley grains with NIV in Hokkaido was caused by the prevailing *F. poae* and *F. crookwellense*.

Fumonisin production by domestic *Fusarium* species in Japan

Fumonisin (FBs) have been implicated in a variety of animal toxicoses, including equine leukoencephalomalacia in horses, porcine pulmonary edema, and experimental liver cancer in rats¹⁾. FBs were found as a metabolite of *F. moniliforme*, a major pathogen of maize and sorghum, in 1988²²⁾. However, no research was done on the natural occurrence of FBs in Japan for a long time. A mycological survey of an FBs-producing *F. moniliforme* was carried out in 1993. Maize stalk dribs formed on the perithecia of *Gibberella* species were sampled at a maize field in Kanuma in Tochigi Prefecture. It was identified as *Gibberella fujikuroi*, the teleomorph state of *F. moniliforme*. Four isolates of *G. fujikuroi* were obtained from ascospores by dissection. Surprisingly, among eight ascospores in an ascus, four were capable of growing on a PDA medium, but the remaining four were not recovered. This experiment was repeated three more times, but the results were the same. As *G. fujikuroi* is a heterothallic fungus, a killer factor may be introduced into the four cells in meiosis. The isolates obtained were tested for the production of FBs, and it appeared that domestic *G. fujikuroi* had good ability to produce FBs on corn grits at levels ranging from 15.7 to 251.7 µg/g, but on rice grains FBs were detected at low concentrations ranging from 2.2 to 4.4 µg/g²³⁾. This was the first report on the production of FBs by a domestic *Fusarium* species in Japan.

As described above, a mycological survey of FB-producing *Fusarium* was not conducted until relatively recently. One reason for the delay in the FBs research is that most Japanese mycotoxin researchers believed that *F. moniliforme* is a pathogen against rice plants and prevalent in rice fields, and therefore ignored the possible presence of FBs-producing *Fusarium* species in maize fields in Japan. Recently, *F. moniliforme* has been shown to be a complex species, including several different species belonging to the section Liseola. Subsequently, in the nomenclature of fungi, *F. moniliforme* was replaced by *Fusarium verticillioides* due to the priority of the latter name²⁴⁾. As a result, the fungal pathogen for maize disease was found to be *F. verticillioides* that produces FBs, and its teleomorph is *Gibberella moniliformis*. The pathogen for rice disease is *Fusarium fujikuroi*, which causes the 'Bakanae' of rice disease in Japan, and produces a plant hormone, gibberellins. Its teleomorph is *G. fujikuroi*.

Experimental model for murine pathogenicity of soil-borne *Fusarium solani*

Recently, *Fusarium* species have emerged as opportunistic fungal pathogens in immunocompromised patients^{25,26)}. *Fusarium solani*, *F. oxysporum*, *F. verticillioides* (= *F. moniliforme*), and *F. proliferatum* cause hyalohyphomycosis^{1,27)} (This term refers to local and systemic infections caused by molds with hyaline and septate hyphae.). In addition, *Fusarium* species are resistant to several chemotherapeutic agents^{26, 28)}. Among the *Fusarium* species, *F. solani* is the most frequent cause of human infection. In Japan, several cases of nail or corneal infections with *F. solani* or *F. oxysporum* have been reported²⁹⁾, but disseminated infection with *Fusarium* species is rare^{30,31)}. However, a number of patients have recently been diagnosed with deep-seated *F. solani* infection.

Japanese scientists reported that soil-borne strains of *F. solani* produce cyclosporins A (CsA) and C

(CsC)³², but no-one has examined CsA production by clinical isolates of *F. solani*. Therefore, I tried to determine whether clinical strains of *F. solani* possess the potential to produce CsA. It appeared that all tested strains derived from clinical sources produced CsA³³. In this trial, I designed a simple analytical method for CsA production of tested strains. As shown in Fig. 3, fungal strains were cultured on YES agar plates at 28 °C for 7 days. Four agar plugs from each plate-culture were cut out of the culture equidistant on the circumference of a 10 mm radius circle from the centre of a Petri dish by using a 7 mm (i.d.) sterile glass tube. Plugs were picked up with a sterile toothpick, transferred into a tared 20 ml vial containing 5 ml of methanol, and then re-weighted to calculate the weight of the agar plugs. After standing for 3 days at room temperature, a 1.25 ml portion of the solution was transferred to a vial, and then evaporated to dryness under N₂. These extracts were analyzed with HPLC equipment for CsA after simple purification³³ (Fig. 4).

CsA and CsC are cyclic undecapeptides with strong immunosuppressive activity; in mammals, they are also nephrotoxic. The immunosuppressive effect is mediated by inhibiting signal transduction, blocking both the transcription of lymphokine genes in T-lymphocytes and also the degranulation of granulocytes³⁴. However, there is no information on the relationship between cyclosporin production and the pathogenicity of *F. solani* in experimental or clinical hyalohyphomycosis or in plant pathology.

A previous report concerning a murine model for *F. solani* infection³⁵ demonstrated that an inoculum size of greater than 1×10^6 colony-forming units (CFU)/mouse was required to cause mortality in CF1 mice. Disseminated infection occurred in mice immunocompromised by an injection of cyclophosphamide. Other studies on experimental *Fusarium* infections have also been reported³⁶⁻³⁹. These experiments were carried out

Fig. 3 Isolation of fungal metabolites

Fig. 4 Purification and HPLC analysis of cyclosporin A

with pathogenic strains of *F. solani* derived from clinical sources, but not with *F. solani* of environmental origin.

In view of the worldwide distribution of *F. solani* as a soil fungus, as well as of the capacity of this fungus to cause human infection, an experimental model to study the toxigenicity and pathogenic potential of soil-borne *F. solani* would contribute significantly to an understanding of opportunistic *Fusarium* infection. Two representative strains of *F. solani* were isolated from soils sampled from a crop field located at Hirosaki (Aomori Pref.) and Isahaya (Nagasaki Pref.) in northern and western regions of Japan, respectively. The *F. solani* strains were characterized by the ability to produce CsA and CsC, and were examined for their pathogenic potential in severe combined immunodeficiency (SCID) and BALB/c mice⁴⁰⁾.

As shown in Table 3, the murine experimental trials demonstrated that no mortality was observed after single dose infection with $0.3\text{--}1.6 \times 10^7$ CFU/mouse in SCID and BALB/c mice. However, 28.6 to 85.7% survival was observed over a 25-day period, depending on the *F. solani* strain and the inbred mouse line used, when mice were infected with $0.8\text{--}1.5 \times 10^6$ CFU/mouse and two days later with $1.2\text{--}1.9 \times 10^6$ CFU/mouse. Death was preceded by renal insufficiency in both kidneys. Interestingly, i.v. injection with heat-killed conidia followed two days later by injecting viable conidia resulted in renal infection in both breeds of mice. *F. solani* isolated from infected organs was more virulent than the original isolate, and 37.5% of BALB/c and 57.1% of SCID mice died after receiving a single dose⁴⁰⁾.

In the murine model experiment, soil-borne *F. solani* strains tested had poor pathogenic potential for mice, but either two successive infective doses or a primary injection with heat-killed conidia followed by a

Table 3. Survival in mice inoculated intravenously with two strains of soil-borne *Fusarium solani*

Trial	Strain	Inoculum dose (CFU/mouse)	Mouse strain	No. mice tested	Median survival time	% survival on day 25	
1	Hirosaki	3×10^6	BALB/c	14	>25	100	
		3×10^6	SCID	14	>25	100	
	Isahaya	1.6×10^7	BALB/c	14	>25	100	
		1.6×10^7	SCID	14	>25	100	
2	Hirosaki	$^a 1.3 \times 10^6 + 1.9 \times 10^6$	BALB/c	7	>25	57.1	($p < 0.05$) ^e
		$^a 1.3 \times 10^6 + 1.9 \times 10^6$	SCID	7	20	28.6	($p < 0.01$) ^e
	Isahaya	$^a 0.8 \times 10^6 + 1.2 \times 10^6$	BALB/c	6	>25	66.7	
		$^a 0.8 \times 10^6 + 1.2 \times 10^6$	SCID	7	>25	85.7	
3	Hirosaki	$^b 1 \times 10^7(\text{conidia/mouse}) + 2.5 \times 10^6$	BALB/c	7	>25	85.7	
		$^b 1 \times 10^7(\text{conidia/mouse}) + 2.5 \times 10^6$	SCID	7	>25	57.1	($p < 0.05$) ^e
4	Hirosaki-amp ^c	0.6×10^6	BALB/c	8	>25	62.5	($p < 0.05$) ^e
	Isahaya-amp ^d	4.3×10^6	SCID	7	18	42.9	($p < 0.01$) ^e

^a Primary injection with viable conidia followed 2 days later with secondary i.v. injection of viable conidia

^b Primary injection with heat-killed conidia followed 2 days later with secondary i.v. injection of viable conidia

^c Hirosaki-amp: strain isolated from a mouse brain after primary passage

^d Isahaya-amp: strain isolated from a mouse kidney after primary passage

^e Difference in survival was analyzed as compared with the same fungal and murine strains in trial 1

single infective dose broke through host defenses in normal and immunoincompetent mice. Mouse passage increased the pathogenicity of two soil-derived *F. solani* strains. Unfortunately, it seemed that medical researchers did not pay any attention to this new finding. This may be due to the lack of additional data to explain why two successive conidial doses are capable of breaking through the murine host defense system. I believe that this experimental result indicates the fragility of the host defense against secondary fungal invasion, and it will contribute to finding ways to prevent fungal infection.

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