

高圧滅菌後微生物相を回復させた土壤に埋没したキュウリ 茎片周辺部におけるPythium butleriとP. zingiberumの行動

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Behaviors of *Pythium butleri* and *P. zingiberum* in and around Cucumber Stem Segments Buried in Recolonized Autoclaved Soil

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Abstract

Behaviors of *Pythium butleri* and *P. zingiberum* in and around the autoclaved cucumber stem segments buried in recolonized autoclaved soil were studied. Oospores of the former germinated around segments, and were more effective in colonization than those of the latter, but no oospore germination was found in *P. zingiberum* under the same environment. Hyphae of *P. butleri* were able to grow more rapidly than those of *P. zingiberum* through the soil. No clear differences in survival in the form of mycelium were found between two fungi. *P. butleri* produced more oospores around the segments than *P. zingiberum*. *P. butleri* survived longer period of time in the colonized segments than *P. zingiberum*.

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Key words: *Pythium* spp., recolonized autoclaved soil.

INTRODUCTION

Pythium butleri Subramaniam causing damping-off of spinach (*Spinacia oleracea* L.)⁷⁾ is a typical plant pathogen in warmer regions. This fungus is considered as synonym of *P. aphanidermatum* (Edson) Fitzp. which is widespread, pathogenic to many plants¹²⁾ and capable of surviving in soil for long period of time¹¹⁾. On the other hand, *P. zingiberum* Takahashi causing rhizome rot of ginger (*Zingiber officinale* Rosc.) and mioga [*Z. mioga* (Thunb.) Rosc.] is a weak parasite in warmer regions and has relatively narrow host range³⁾. This fungus is detected only in soil with host growing, and may not be able to survive in soil for long period of time^{4,5)}.

These two species are morphologically and taxonomically related¹²⁾ but probably different ecologically. In this study, we compared their behaviors in and around cucumber stem segments in soil. Part of the work has been reported elsewhere¹³⁾.

MATERIALS AND METHODS

Fungal isolate and media. *P. butleri* [UOP 286 (=IFO 31214)] and *P. zingiberum* [UOP 8001-2 (=ATCC 46851, CBS 21782)] were used in all experiments. The origin and characteristics of these isolates have been defined^{6,7)}. The fungi were maintained as described elsewhere²⁾. The media used for production and direct germination of oospores have already been described⁸⁾. A plain water agar (20 g agar, Wako Pure Chem. Ind., Ltd., Osaka, 1,000 ml distilled water, pH 5.0) was used for fungal detection.

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Soil. All colonization experiments were conducted in clay loam soil (pH 6.1). The soil passed through a 9 mesh-sieve was sterilized by autoclaving, and then placed in a wooden box (40×70×15 cm) on a bench under glasshouse conditions (8–36 C) for more than one month to recolonize microorganisms before use. Tap water was supplied at irregular intervals and any weeds were removed.

Substrate for colonization. The autoclaved stem segments (5 mm long, 3 mm in diam.) of cucumber (*Cucumis sativus* L. cv. Santo Suyu No. 2) harvested from the 2-month old plants grown in the glasshouse were used as substrates for colonization since they were equally infected in soil with two fungi employed (Ichitani, unpublished data). They were stored in distilled, deionized water at 6 C in the dark until used. The segments harvested at the same time were used for the same series of the experiments.

Oospore suspension. This was prepared by the method described previously^{6,8)}.

Direct germination of oospores on culture medium or in soil. Oospore germination was examined on Bacto-CMA[®]. This was also tested in soil by essentially the same method reported previously^{6,9)}. About 1.0×10^3 oospores adhered to 10 mm² screen (10 μ m pore) were inserted into soil in a 100 ml Erlenmyer flask at the depth of about 15 mm and incubated at 25 C for 24 hr in the dark with or without one segment just above the screen. After removing adhered soil from the screen, oospores were stained with 0.1% cotton blue in lactophenol and the germination percentage was calculated with mature oospores incorporated.

Colonization of segments in soil. All colonization experiments were performed in soil in 100 ml Erlenmyer flasks with silicone plug. Soil (equivalent to 40 g oven dried soil) in each flask was mixed with oospore suspension (4 ml) at different inoculum densities. Twenty segments were placed at random in the soil. After incubation at 25 C for 3 days, all segments were recovered, washed with tap water, placed between filter papers to dry, and plated on the plain water agar to detect fungal growth.

Survival in the form of mycelium and formation of oospores around segments. Each fungus was inoculated centrally onto Bacto-PDA plate with eight 10 mm² sterile nylon screens (10 μ m pore) on the periphery. The screens covered uniformly with hyphae were removed from the agar surface after incubation at 25 C for 3 days for *P. butleri* and 5 days for *P. zingiberum*. Hyphae were sandwiched between two screens and buried in soil in the same manner as described in oospore germination test.

After incubation at 25 C in the dark for a given period of time, screens with hyphae were removed from the soil, washed with sterile distilled water, and active hyphae containing protoplasm were counted. Relative value of active hyphal strands/40 microscopical fields/screen was calculated at different time intervals. Number of oospores produced on each screen was also counted. These screens were then transferred onto newly prepared Bacto-CMA supplemented with 40 ppm streptomycin to determine viability of mycelia.

Effect of burying period on isolation. Fifty segments buried for 2–70 days in soil with different density of propagules or oospores (180 g of soil mixed with 20 g of infested soil) were plated on plain water agar. Fungal growth from the segments was detected as described in the above colonization test.

In every experiment, the water content of the soil was adjusted to about 30% (which is equivalent to matric potential of –150 mb) to prevent indirect germination of oospores.⁶⁾ All experiments were repeated 2–6 times with 2–5 replications.

RESULTS AND DISCUSSION

Relation of oospore density to colonization

In the Cambridge method^{1,10)} applied for *P. butleri* and *P. zingiberum* using autoclaved cucumber stem segments, no differences in competitive saprophytic ability (CSA) were found between two fungi. The CSA was, then, examined quantitatively using oospores as an inoc-

ulum. *P. butleri* was able to colonize at 1.0×10^1 oospores per gram of oven dried soil (OPG), whereas *P. zingiberum* could not consistently colonize even at 1.0×10^4 OPG. When tested using an appropriate oospore density of these two fungi which gave about the same degree of CSA, OPG in *P. zingiberum* was higher than those in *P. butleri* by about 100 times.

Relation of oospore germination to colonization

The percentage of oospore germination in *P. zingiberum* is too low (below 10%) to examine CSA. In addition to the trials to induce the germination reported previously⁶⁾, some other treatments were tested unsuccessfully. Then, the relation of oospore germination to colonization was determined (Table 1). The OPG needed for colonization may depend on oospore germination in soil.

Differences in rate of mycelial growth in soil

When cucumber stem segments were placed on different amounts (5–30 g) of soil over fungal cultures of Bacto-CMA in 9-cm petri dish, colonization of the segments by *P. butleri* was higher than that by *P. zingiberum*. This suggested that hyphae of *P. butleri* can grow more rapidly than *P. zingiberum* through the soil.

Differences in survival in the form of mycelium

No clear differences in survival in the form of mycelium were found between *P. butleri* and *P. zingiberum*.

Oospore formation around segments

Oospore formation was observed around segments in *P. butleri*, but not in *P. zingiberum* (Table 2).

Effect of burying period on isolation

Finally, two fungi were compared for survival after colonization in soil. *P. butleri* was isolated from almost all segments throughout the experiment, whereas *P. zingiberum* was not so and rate of isolation decreased with the incubation proceeded (Fig. 1). Similar results were obtained when segments were buried for 2 days in the above soil previously incubated for var-

Table 1. Relation of oospore germination of *P. butleri* and *P. zingiberum* to colonization of cucumber stem segments in soil

Oospores ^{a)}	Germination or colonization (%)	
	<i>P. butleri</i> (UOP 286) ^{b)}	<i>P. zingiberum</i> (UOP 8001–2)
Germinated		
On Bacto-CMA ^{c)}	29.6	7.2
In Soil ^{d)}		
With a cucumber stem segment	9.9	0.0
Without a cucumber stem segment	0.3	0.0
Incorporated/g oven dried soil		
1.0×10^4	15–35 ^{e)}	0
1.0×10^3	— ^{f)}	0
1.0×10^2	0–10	—
1.0×10^1	0	—
Not incorporated	0	0

a) Oospores harvested 24 and 21 days after inoculation for *P. butleri* and *P. zingiberum*, respectively.

b) Isolate no.

c) At 25 C for 22–24 hr just before incorporation.

d) At 25 C for 24 hr after incorporation.

e) The range of fluctuation indicates that the data varied with the duplicates. One numeral indicates that there was no fluctuation.

f) Not tested.

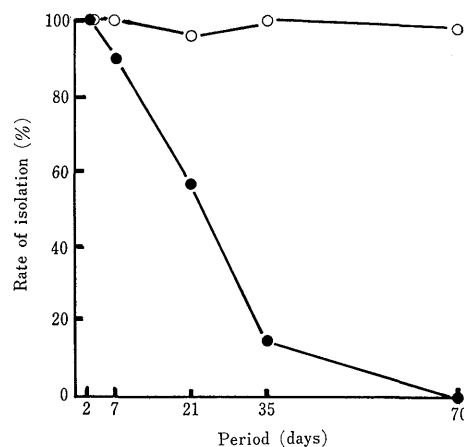
Table 2. Oospore formation of *P. butleri* and *P. zingiberum* around cucumber stem segment in soil

Period (days)	<i>P. butleri</i> (UOP 286) ^{a)}		<i>P. zingiberum</i> (UOP 8001-2)	
	Buried ^{b)}	Not buried	Buried	Not buried
0	0 (0) ^{c)}	0 (0)	0 (0)	0 (0)
1	0 (2)	0 (0)	0 (0)	1 (0)
2	7 (0)	0 (0)	0 (0)	0 (0)
4	4 (3)	0 (0)	0 (0)	0 (0)
8	5 (4)	0 (0)	1 (0)	0 (0)
12	0 (3)	0 (0)	0 (0)	0 (0)
16	1 (3)	0 (0)	0 (0)	0 (0)
20	0 (1)	0 (0)	0 (0)	0 (0)
24	2 (0)	0 (0)	0 (0)	0 (0)

a) Isolate no.

b) One cucumber stem segment/flask.

c) The number of oospores was counted in 40 microscopic fields (500×) with duplicate screens. (): replication.

Fig. 1. Isolation of *P. butleri* and *P. zingiberum* from soil by cucumber stem segments buried for different periods of time. ○: *P. butleri* (UOP 286), ●: *P. zingiberum* (UOP 8001-2).

ious periods of time. The results showed that *P. butleri* was more suitable for survival in soil than *P. zingiberum*.

The present experiments may indicate that differences in colonization of the segments by both fungi are due to oospore germination and mycelial growth around segments, and that differences in survival after colonization depend on oospore formation inside the segments.

This study was conducted using only one isolate for each species since it is considered to be typical from the viewpoints of morphological, cultural and pathological characteristics (Ichitani, unpublished data). Further studies are, however, needed to be done with more isolates before final conclusion can be drawn.

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和 文 摘 要

鮫島信隆・一谷多喜郎：高圧滅菌後微生物相を回復させた土壌に埋没したキュウリ茎片周辺部における *Pythium butleri* と *P. zingiberum* の行動

高圧滅菌したキュウリ茎片を高圧滅菌後微生物相を回復させた土壌に埋没し、その周辺部における *Pythium butleri* と *P. zingiberum* の行動を比較した。*P. zingiberum* に比べ、*P. butleri* の卵胞子は茎片の周辺部で高い発芽率を示した。また、*P. butleri* の菌糸は土壌中をより速く伸長し、より少数の卵胞子によっても茎片に定着することができた。したがって、両菌のキュウリ茎片への定着率の違いは、土壌に埋没した茎片周辺部における卵胞子の発芽能と菌糸の伸長速度の差に起因すると考えられた。さらに、*P. zingiberum* に比べ、*P. butleri* は定着した後の茎片から長期にわたって分離され、土壌中におけるより高い生存能が認められた。*P. butleri* は茎片周辺部でより多くの卵胞子を形成した。しかし、両菌菌糸の土壌中における生存能には差が認められなかった。したがって、土壌中に埋没されたキュウリ茎片に定着した後に示す両菌の生存能の差異は、同茎片内における卵胞子形成能の違いによると推察された。