

土壌中における *Pythium zingiberum* 繁殖体の定量および観察のための選択培地

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
著者名	清水,時哉 一谷,多喜郎
発行元	日本植物病理學會
巻/号	50巻2号
巻号補足	
掲載ページ	p. 289-293
発行年月	1984年4月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council
Secretariat



Selective Medium for Quantitative Detection and Observation of Propagules of *Pythium zingiberum* in Soil

Tokiya SHIMIZU* and Takio ICHITANI**

清水時哉*・一谷多喜郎** : 土壌中における *Pythium zingiberum*
繁殖体の定量および観察のための選択培地

Key Words : Selective medium, *Pythium zingiberum*, quantitative detection, observation, soil.

The soil dilution plate method has relatively low sensitivity for detecting *Pythium zingiberum* in soil¹⁾ and sometimes difficult to find its propagules. This paper deals with a new selective medium to improve sensitivity and to find the propagules more clearly. Part of the work has been reported elsewhere²⁾.

The isolates of *P. zingiberum* UOP237, UOP252, UOP6656 causing rhizome rots of ginger and mioga^{3,4)} were mainly used. Both artificially (AI) and naturally infested (NI) soils were compared for quantitative detection and observation of propagules of this fungus. AI soil was prepared by mixing a known density of the propagules with 0.14 % water agar containing sandy loam soil (autoclaved unless otherwise stated). NI soil was collected from the inside of plastic house where rhizome rot of ginger was unusually prevalent and kept in plant containers, either concrete cylinders or pots⁵⁾. The medium of a routine dilution plate method⁶⁾ was compared with the new medium regarding the propagules detected per gram oven dry soil (PPG) and/or their recovery percentages (R%). Experiments were repeated at least twice.

Some experiments were performed in order to devise the new selective medium by reducing the amount of rose bengal in the routine medium⁶⁾ and by adding antibiotics which has preliminarily confirmed to show any inhibitory effects on mycelial growth of *P. zingiberum* but toxic to soil bacteria. Results obtained from AI soil indicated that the sensitivity of detection increased slightly in a medium modified by reducing the rose bengal from 33 to 20ppm, increasing streptomycin (Meiji) from 40 to 100 ppm and adding 100 ppm vancomycin (Sigma). Similar results were obtained from NI soil. This

* Present address : Nagano Agricultural Experiment Station, Suzaka, Nagano 382, Japan 現在 :
長野県農業試験場

** College of Agriculture, University of Osaka Prefecture, Sakai, Osaka 591, Japan 大阪府立大学
農学部

1) Ichitani, T., Tanaka, Y. and Tagami, Y. (1980). Ann. Phytopath. Soc. Japan 46 : 86-87 (Abstr.).
2) Shimizu, T. and Ichitani, T. (1983). *Ibid.* 49 : 99 (Abstr.). 3) Ichitani, T. and Shinsu, T. (1980).
Ibid. 46 : 435-441. 4) Ichitani, T. and Chikuo, Y. (1980). *Ibid.* 46 : 539-541. 5) Ichitani, T. and
Shimizu, T. (1984). Bull. Univ. Osaka Pref., Ser. B 36 : 15-19. 6) Ichitani, T. (1981). Proc. Kansai
Pl. Prot. Soc. 23 : 60 (Short com.).

Starch-Peptone-Asparagine Medium

KH ₂ PO ₄	1.0	} g/l distilled water; stored at 4 C and used up within 1 month; pH 6.
MgSO ₄ ·7H ₂ O	0.5	
peptone	5.0	
L-asparagine	0.27	
starch	9.0	

Selective medium for dilution plate method

— diluted the Starch-Peptone-Asparagine Medium to 32 times
and supplemented with:

rose bengal	20 ppm	} added before autoclaving.
agar	2 %	
benomyl (Du Pont)	20	} ppm; added after autoclaving.
pimaricin (Torii)	80	
streptomycin sulphate (Meiji)	100	
vancomycin (Sigma)	100	

Fig. 1. Composition and preparation of the new selective medium.

Table 1. Comparative detection of *Pythium zingiberum* in artificially infested unautoclaved field soil by new selective medium under different incubation temperatures^{a)}

Selective medium	Incubation temperature (C)	Propagules/g oven dry soil	
		Exp. 1	Exp. 2
New selective medium	26	19.4	21.8
	30	48.4	72.6
	34	87.1	130.7
Routine medium	26	—	9.7
	30	7.3	26.6
	34	16.9	60.5

a) Soil used was free from *P. zingiberum*. Inoculum density in Exp. 1: 2.5×10^5 hyphae mixed with 9.0×10^4 oospores/g oven dry soil. Inoculum density in Exp. 2: 4.8×10^5 hyphae mixed with 8.1×10^4 oospores/g oven dry soil. New selective medium: see Fig. 1. —: Not detected.

modified medium was also shown to be superior to the routine medium for observing propagules in NI soil (Plate I) and for rapid identification⁷⁾. Amending this modified medium with amino acids or carbon sources previously selected for the mycelial growth of *P. zingiberum*, the dilution plate method was again applied for AI soil. Sensitivity was increased further, especially with L-asparagine or starch. Similar results were obtained from NI soil.

7) Shimizu, T. and Ichitani, T. (1982). Ann. Phytopath. Soc. Japan 48: 691-694.

From the above results, the new selective medium was devised (Fig. 1). With this medium, detective sensitivity of *P. zingiberum* increased in higher incubation temperature (Table 1), but the propagules in soil were easily detected only on the new medium at 34 C (Plate II). This is probably due to high sensitivity of soil bacteria to the antibiotics at higher temperature. Moreover, incubation at 34 C, at which most *Pythium* spp. are unable to grow⁸⁾, increase specificity of the present method for *P. zingiberum*. The best incubation time on the new medium was 24 hr for both detection and observation of propagules.

The antibiotic "Agrimycin" (Daito Pfizer) gave poor results, probably due to the toxicity of oxytetracycline to *P. zingiberum*. Since this fungicide is easily obtainable and has been used for isolating other *Pythium* spp. from soil⁹⁻¹¹⁾, its application to *P. zingiberum* needs further study.

Sensitivity of the new medium increased about one order of magnitude over that of the routine medium (see Table 1). The lower limit of detection was estimated as 10^2 PPG and R% of propagules was calculated by the new medium at 34 C as the values between 0.025 and 0.129, equivalent to 10^3 – 10^5 PPG (see Table 1). The difference between the lower limit of detection and R% is probably due to lower percentage (0.5–6.5 %) of oospore germination of *P. zingiberum* on this medium. The uniform lower percentage of germination on several agar media, including the new one, may also indicate that there is at least no inhibitory action of the new medium on oospore germination of this fungus.

The new medium should be evaluated in ecological studies of *P. zingiberum* and the diseases it causes.

The authors are indebted to Prof. T. Inouye, University of Osaka Prefecture, and to Dr. D.J. Stamps, Commonwealth Mycological Institute, U.K., for their critical readings of the manuscript.

(Received December 19, 1983)

8) Middleton, J. T. (1943). Torrey Bot. Club Mem. 20 : 1–171. 9) Kageyama, K. and Ui, T. (1980). Ann. Phytopath. Soc. Japan 46 : 542–544. 10) Kageyama, K. and Ui, T. (1981). *Ibid.* 47 : 313–319. 11) Kageyama, K. and Ui, T. (1982). *Ibid.* 48 : 308–313.

Explanation of plates

Plate I

1-4. The medium modified by reducing the amount of rose bengal, increasing streptomycin, and adding vancomycin (1,2) which was shown to be superior to the routine one (3,4) for observing propagules in naturally infested soil.

1,3: Filamentous sporangium-like structures

2: oospore

4: undetermined

Arrows indicate the nature of propagules.

Plate II

1-6. The new selective medium which was easily detected the propagules at 34 C in artificially infested unautoclaved field soil (3) as compared with the routine one (6).

1-3: Detected on the new selective medium.

4-6: Detected on the routine medium.

1,4: Detected at 26 C

2,5: Detected at 30 C

3,6: Detected at 34 C

Plate I

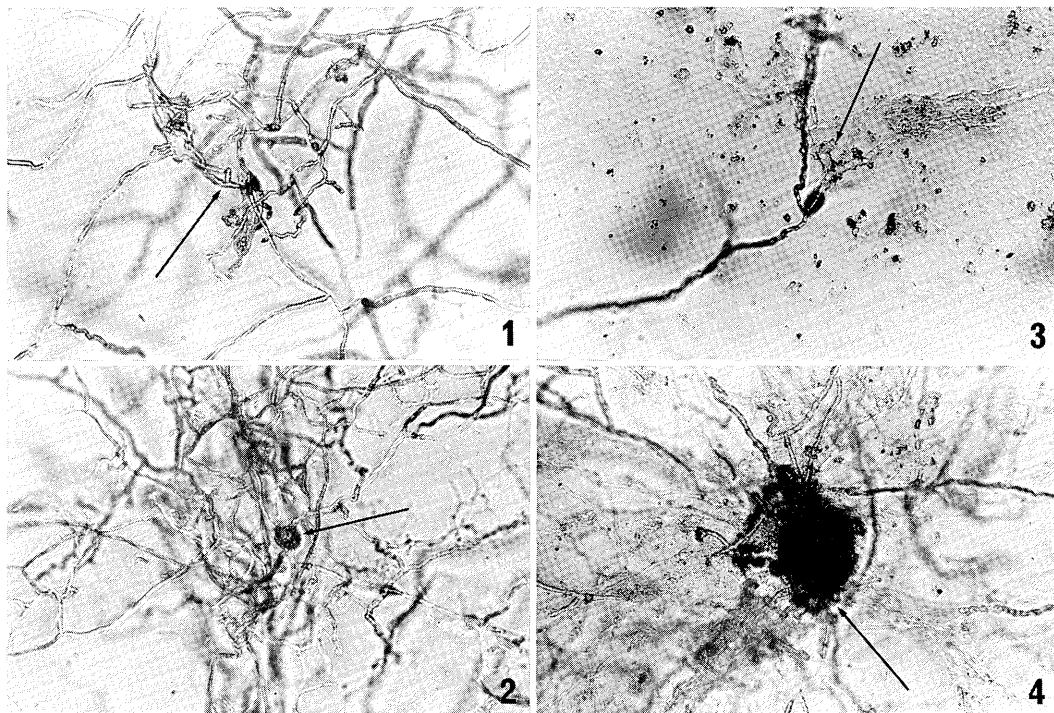


Plate II

