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Studies on *Pythium* spp. in Korea
(I) Preliminary taxonomic and physiological studies

Eung Kwon Lee, Young Hee Lee, Jae Dang Yoo, Peter G. Long

Institute of Agricultural Sciences, O.R.D., Korea

韓國의 *Pythium* spp. 에 關한 硏究
(I) 分類 및 生理學的 基礎硏究

李應權, 李永熙, 柳在塘, Peter G. Long

農村振興廳 農業技術硏究所

Abstract: Three species of *Pythium* previously not recorded in Korea were found during 1975. *Pythium spinosum* Sawada was isolated from diseased cabbage seedlings, *P. myriotylum* Dreschl. was isolated from kidney bean and *P. butleri* Subramanium from cucumber, spinach, red bean and radish. Pathogenicity of isolates of *P. butleri* and *P. myriotylum* was confirmed in pathogenicity tests but *P. spinosum* appeared to be non-pathogenic.

Several isolates failed to sporulate satisfactorily on cornmeal agar and some produced degenerate oogonia after sub-culturing on this medium. Sunflower seed agar was found to be a suitable alternative medium.

The validity of previous records of *P. debaryanum* Hess in Korea is discussed.

Introduction

The only species of *Pythum* recorded from Korea is *P. debaryanum*. It is listed in the 1972 host index of plant diseases of Korea as the cause of damping-off disease of tobacco, cucumber and melon. It has also been found in soil plates made with soil taken from ginseng seed beds.

This survey of *Pythium* diseases was initiated for two reasons;

a) *Pythium* spp. are common soil fungi and it is most unlikely that only one species occurs in Korea.

b) Many of the reports of *P. debaryanum* in other countries have subsequently been found to be mis-identifications. Verification of the Korean reports is needed.

During the course of this work it was found that *Pytium* isolates degenerated when kept on cornmeal agar. Alternative growth media were therefore investigated.

Materials and Methods

a) Isolation of pathogens

The advance margin of the diseased plant material was removed and washed in running water to remove soil and debris. These disease lesions were surface-sterilised for 2 minutes in freshly made 1% sodium hypochlorite solution and plated out on cornmeal(CM)or water agar(WA) contain-

ing streptomycin. *Pythium* spp. grew rapidly on these media and could be isolated after 24-28 hrs. incubation at room temperature (23~39°C).

b) Morphology

Colonies growing on sunflower seed agar were mounted in water or glycerine and spore measurements were made at a magnification of 600 x using eyepiece graticule. Photomicrographs were taken with an Olympus PM7 unit.

c) Pathogenicity tests

Soil was sterilised by autoclaving at 15 lb/squ. in. for 15 minutes.

Sand-cornmeal cultures of the fungi were prepared by placing a mixture of 98 g sand and 2 g finely ground cornmeal in each of a series of 250 ml flasks. Sufficient water was added to obtain a wet medium without waterlogging. The flasks were plugged with cottonwool, autoclaved and inoculated when cool. The flask cultures were incubated for 7 days and then the inoculum was mixed with sterilised soil at the rate of 20 g sand-cornmeal culture to 550 g soil. Seeds were sown immediately after inoculation. Three inoculated and three uninoculated pots were used for each isolate tested.

Twelve ungerminated seeds or diseased seedlings were removed from each set of pots several days after inoculation in order to confirm pathogenicity by reisolation of the pathogen. These seeds were washed in clean water, surface-sterilised for 2 minutes in 1% sodium hypochlorite and plated out on cornmeal or water agar. Percentage motality was recorded 3 weeks after planting.

d) Physiological tests

Potato dextrose, cornmeal and water agar were made using standard commercially prepared powders. Sunflower seed agar was prepared by grinding shelled sunflower seeds in a pestle and mortor and boiling 15 g of the pulp in a litre of water for 15 minutes. The mixture was then strained through cheese cloth and 17 g of agar powder was added before sterilisation in the autoclave.

The effect of cholesterol was tested by sprinkling a small quantity (about 0.1 g) on the surface of agar plates.

Results

a) Taxonomy and morphology

Most surveys were carried out in the area around Suweon during the summer months. Six *Pythium* isolates were obtained from diseased seedlings of various vegetables and one from cucumber fruit (Table 1). Three species were represented in the seven isolates. Isolates of *P. butleri* Subramanium had relatively course hyphae, lobulate sporangia and aplerotic oogonia with one antheridium per oogonium. This was he commonest species. The isolate of *P. myriotyum* Dreshl (75/205) produced larger oogoniaoth anhose of *P. butleri* and there were many anth eridia to each oogonium. Isolate 75/55 (*P. spinoum* Hesse) was very distinctive because of thspiny oogonia. However, in some dishes, particularly those of sub-cultures growing on cornmeal agar, there were few spiny oogonia.

Table 1. Details of *Pythium* isolates

Isolate No.	Date isolated	Host and disease	Locality
75/55	22/5/75	Cabbage seedling blight	Cheonhodong
75/122	24/7/75	Cucumber fruit rot	Suweon
75/165	27/8/75	Spinach seedling blight	Suweon
75/187	5/9/75	Red bean seedling blight	Suweon
75/195	8/9/75	Spinach seedling blight	Suweon
75/205	12/9/75	Kidney bean seedling blight	Suweon
75/207	27/8/75	Radish seedling blight	Suweon

Table 2. Morphology of *Pythium* species

Isolate No.	75/122	75/165	75/187	75/195	75/207	75/205	75/55
Hyphal diam. (u)	7.1	6.1	5.0	6.0	7.1	4.9	2.9
Sporangial diam. (u)	15.6	13.7	17.6	15.2	15.4	13.9	16.0
Oogonial diam. (u)	25.7	26.4	24.6	25.9	26.9	32.7	17.2
Oospore diam. (u)	21.1	22.3	21.9	21.9	21.9	29.3	16.4
Antheridial diam. (u)	10.7	11.0	10.7	11.1	11.3	4.9	4.7
No. of antheridia per oogonium	1	1	1	1	1	9.4	1.3
Identification	*P. butleri* Subramaniam					*P. myriotylum* Dreschl	*P. spinosum* Sawada

b) Pathogenicity tests

The results of pathogenicity tests are shown in Table 3. All isolates of *P. butleri* were highly pathogenic and caused considerable mortality of seedlings in inoculated pots. Each isolate was readily re-isolated when diseased seedlings were surface sterilised and plated out on agar.

The isolate of *P. myriotlum* (75/205) was highly pathogenic to kidney bean seedlings. The isolate of *P. spinosum* (75/55) appeared to be non-pathogenic.

Table 3. Mortality of seedlings in inoculated pots expressed as a percentage of that in uninoculated pots

Isolate	Percentage mortality
75/55	0
75/122	100
75/165	100
75/187	48
75/207	92
75/205	100

c) Physiological tests

Three isolates (one of each species) were used to asses the growth of *Pythium* spp. on various media (Table 4). Isolates 75/55 (*P. spinosum*) and 75/122 (*P. butleri*) grew fastest on potato dextrose agar but 75/205 (*P. myriotylum*) grew fastest on cornmeal agar. Vegetative growth of all three isolates was slowest on water agar. The addition of cholesterol had little effect on mycelial growth of these fungi except on water agar where growth of 75/55 and 56/205 was markedly less in the presence of cholesterol.

Isolate 75/55 produced more sporangia on sunflower seed agar than on the other media and addition of cholesterol increased the production of sporangia on this medium. There were more sporangia of isolate 75/122 on cornmeal than agar on the other media but addition of cholesterol did not appear to influence sporangial production on any of the media. Few sporangia of isolate 75/205 were formed on any medium, with or without cholesterol (Table 5).

Table 4. Fffect of different agars and cholesterol on vegetative growth of 3 *Pythium* isolates measured as the colony diameter (mm) after 24 hours incubation at room temperature (23.5~29.0°C)

	75/55	75/122	75/205
S	49	52	44
P	58	72	46
C	48	58	57
W	43	42	24
S+CH	45	53	45
P+CH	64	68	54
C+CH	50	55	57
W+CH	31	34	26

S=sunflower seed agar
P=potato dextrose agar
C=cornmeal agar
W=water agar
CH=cholesterol

Table 5. Effect of different media and cholesterol on the number of reproductive structures formed by *Pythium* isolates after 4-days incubation. The table shows the number of reproductive structures counted per field of view at 150× magnification (mean of 3 replicates per observation)

	75/55		75/122		75/205	
	Oogonia	Sporangia	Oogonia	Sporangia	Oogonia	Sporangia
S	4.3	1.0	94.0	3.0	13.3	0
P	0	0	36.3	3.3	1.0	0
C	1.7	0	20.3	11.0	0	0
W	1.0	0	14.3	1.7	0.3	+
S+CH	9.3	5.7	103.3	2.7	13.0	0
P+CH	0	0	45.0	4.0	1.3	0
C+CH	2.0	0.7	14.0	12.7	0	0
W+CH	0.7	0	9.0	2.0	0.3	+

+=No sporangia in the three fields of view but a few were present in other parts of the dish.

The type of growth medium influenced the production of oogonia more than it did that of sporangia. All isolates produced more oogonia on sunflower seed agar than on any other medium. Addition of cholesterol did not markedly affect the production of oogonia of any isolate on any medium with the sole exception of isolate 75/55 growing on sunflower seed agar where addition of cholesterol singifi-cantly increased the production of oogonia ($p=0.01$) and sporangia (0.05).

Discussions

Three species of *Pythium* previously unrecorded in Korea were found in this survey. *P. spinosum* and *P. butleri* are known to be widely distributed geographically but *P. myriotylum* is common only in warm climates(CMI description no. 118). Korea is hot in the summer months but extremely cold in the winter. *P. myriotylum* therefore must be capable of surviving sub-zero conditions for sometime even though it grows best at high temperatures. It would be interesting to survey other countries with extremes of climate (eg. Japan and southern Canada) to determine whether this species is present there also.

P. debaryanum was the only species of *Pythium* recorded from Korea prior to this survey. It was recorded on tomato, cucumber, melon and from ginseng seed beds (Anon. 1972). This range of records would suggest that it is a common pathogen in Korea yet it was not found in the present work. One possible explanation is that it does not occur in the areas surveyed. Alternatively it may be an active pathogen only in the spring and autumn when temperatures are lower. A third possibility is that one of the species found in this work was previously mis-identified as *P. debaryanum*. *P. spinosum* formed normal sporangia or cornmeal agar but the oogonia were sometimes without spines. In such cases it is conceivable that *P. spinosum* could have been mistaken for *P. debaryanum*. Further work must be carried out before the presence of the latter in Korea can be confirmed.

Pythium cultures kept on cornmeal agar often produced abortive sex organs or even failed to produce any at all. The failure of *Pythium* spp. to reproduce sexually on cornmeal, potato dextrose and similar agars is well known and usually hemp seed extract agar or similar agar containing sterols is needed to stimulate the mating reaction (Hendrix and Campbell, 1973; Hendrix and Papa, 1974). Hemp seeds are not readily available in Korea but sunflower seeds are common and are known to contain sterols. The three *Pythium* spp. used in this work grew well and formed typical sexual structures on sunflower seed agar. Addition of cholesterol to agar media

as a sterol source was not generally beneficial.

Acknowledgements

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摘 要

只今까지 韓國에는 담배, 오이, 참외, 人參 等에서 立枯病을 이르키는 *Pythium debaryanum*을 報告한 것 뿐이였으나 1975年 著者들은 3種의 未記錄 *Pythium* 菌을 同定할 수 있었다. *P. spinosum* Sawada는 양배추에서 *P. myriotylum* Drechl은 강낭콩에서 그리고 *P. butleri* Subr는 오이, 시금치, 팥, 무우等의 寄主에서 分離하였다. *P. myriotylum*과 *P. butleri*는 病原性이 認定되었으나 *P. spinosum*은 病原性이 없는 것으로 나타났다.

Cornmeal agar 培地에서는 大部分의 分離菌이 完全한 子實體形成이 되지 않았으며 몇개의 分離菌은 같은 培地에서 繼代培養함에 따라 退化된 子實體를 形成하였다. 그러나 해바라기 種子를 利用한 培地에서는 모든 分離菌이 滿足할만한 子實體를 形成하였다.

References

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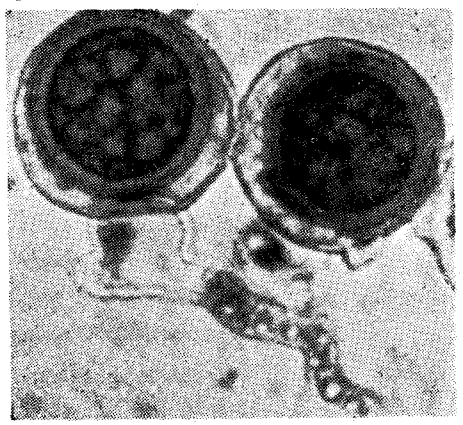
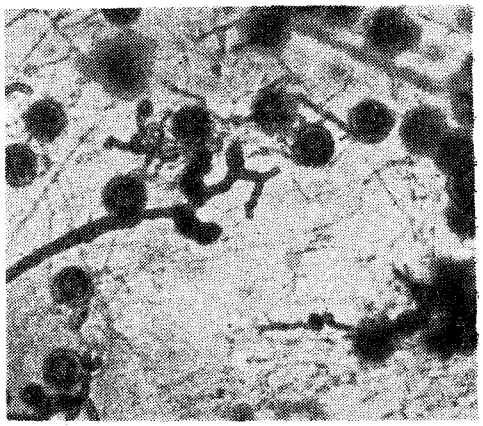
Hendrix F. F. and K. E. Papa. (1974) : Taxonomy and genetics of *Pythium*. *Proceedings of the Amer. Phytopathological Soc.* **1**:200-207.

Plates

A

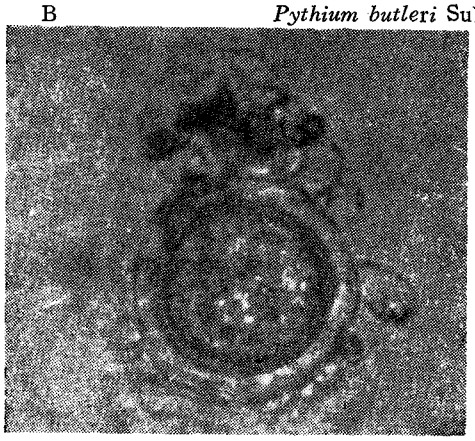


Oogonia with antheridium (400×)

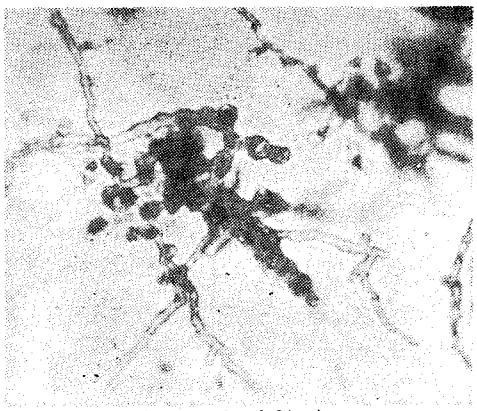


Sporangia and oogonia(100×)

B *Pythium butleri* Subramaniam

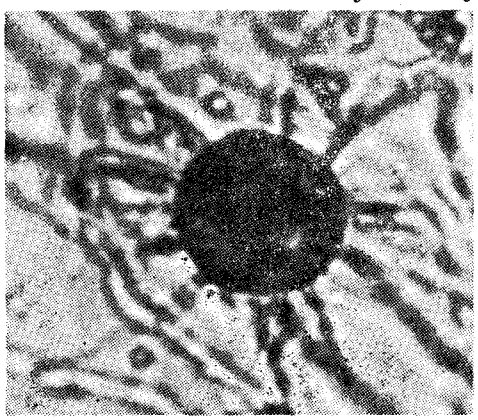


Oogonium with antheridia (400×)

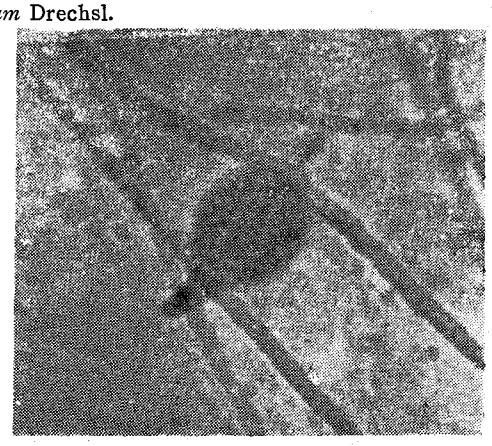


Sporangia (100×)

C *Pyhthium myriotylum* Drechsl.



Oognium with antheridium (400×)



Spherical sporangium (400×)

Pythium spinosum Sawada