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Effect of culture origin on chemical stimulation of sexual reproduction in *Phytophthora* and *Pythium*

Hau Wu[1], Xiau-Bo Zheng[1], and Wen-Hsiung Ko[2,*]

[1]*Department of Plant Protection, Nanjing Agricultural University, Nanjing 210095, P.R. China*

[2]*Department of Plant and Environmental Protection Sciences, Beaumont Agricultural Research Center, University of Hawaii at Manoa, Hilo, Hawaii 96720-4037, USA*

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Abstract. Lecithin washed with NaCl solution was stimulatory to oospore formation of *Phytophthora boehmeriae*, *P. sojae*, *P. cactorum*, and *Pythium aphanidermatum*. Commercial glycerides of palmitic acid with 99% purity were found to contain a substance inhibitory to oospore formation. Purification with florisil column chromatography was more effective in removing the inhibitory substance than thin-layer chromatography or NaCl washing. After purification, monopalmitin, dipalmitin and tripalmitin were found to be stimulatory to the sexual reproduction of *Phytophthora* for the first time. These compounds were stimulatory to oospore formation of *P. boehmeriae* and *P. sojae* but not *P. medicaginis*, and were stimulatory to isolate S317 of *P. sojae* but not isolate CN-1 of the same species. *Pythium aphanidermatum* cultured on basal medium grew sparsely but steadily and produced oospores after being transferred to nutrient-free water agarose. Extracts from mycelia of *P. boehmeriae, P. cactorum*, and *P. sojae* grown in liquid basal medium were stimulatory to their own sexual reproduction. Results support the hypothesis that pythiaceous fungi can synthesize substances needed for sexual reproduction but require a stress factor to trigger the process.

Keywords: Lecithin; Glycerides; *Phytophthora* spp.; *Pythium* spp.; Sexual reproduction.

Introduction

Phytophthora and *Pythium* species are two of the very destructive groups of plant pathogens in the world (Van der Plaats-Niterink, 1981; Erwin and Ribeiro, 1996). These two groups of fungi are classified in the family Pythiaceae of the Oomycetes, which is excluded from the traditional "true fungi" of the kingdom Myceteae. The family is instead included with brown algae in the kingdom Chromista because the major part of their life history is diploid whereas other fungi are haploid (Erwin and Ribeiro, 1996).

Sexual reproduction in *Phytophthora* and *Pythium* is very important in the life cycle of these fungi because it provides not only a means of propagation and survival in nature but also a potential source of genetic variation. In 1964, scientists from several institutes independently reported that sterols are required for sexual reproduction in *Phytophthora* (Harnish et al., 1964; Elliott et al., 1964; Hendrix, 1964; Leal et al., 1964) and *Pythium* (Haskin et al., 1964; Hendrix, 1964). The alleged essentiality of sterols for sexual reproduction in pythiaceous fungi has been frequently cited as factual (Smith and Berry, 1974; Barnett, 1976; Webster, 1980).

During the 1980's, it was found that lecithin was also stimulatory to the sexual reproduction of *Phytophthora cactorum* and *Pythium aphanidermatum* (Ko and Ho, 1983; Ko, 1985, 1986). Moreover, *Phytophthora parasitica* and *Phytophthora capsici*, whose sexual reproduction was not responsive to sterols, were also stimulated by lecithin to produce oospores (Ko, 1985; Jee et al., 1997). According to the criteria of essentiality, an essential substance can not be replaced by any other substance (Bidwell, 1974; Naggle and Fritz, 1976). It was, therefore, concluded that sterols are not essential for sexual reproduction in pythiaceous fungi (Ko, 1988, 1998). Our subsequent study revealed the common presence of inhibitory substances in the highly purified commercial compounds (Jee et al., 1997; Jee and Ko, 1997). After removal of these inhibitory substances, a number of fatty acids and related compounds were found to be stimulatory to the oospore formation of *P. cactorum*, and some of them were also stimulatory to *P. parasitica* (Jee et al., 1997; Jee and Ko, 1997).

Prior to our discovery of the stimulatory effect of non-sterol substances on oospore formation, the failure of pythiaceous fungi to produce sexual progeny on basal medium was attributed to their inability to synthesize the substances required for sexual reproduction (Elliot, 1983; Hendrix, 1970; Nes, 1987). This hypothesis was subsequently refuted as extracts from mycelia of *P. cactorum* grown in liquid basal medium were shown to be capable of inducing oospore formation in *P. cactorum* and *P. parasitica* (Jee and Ko, 1998). Our results show that *P. cactorum* can synthesize substances needed for sexual reproduction, but it requires a stress factor, such as nutrient deprivation, to trigger the process.

*Corresponding author. Fax: +1-808-974-4110; E-mail: kowh@hawaii.edu

Since only three species of *Phytophthora* and one species of *Pythium* were used in the above study, it was not known if other species or isolates of the same species of pythiaceous fungi would respond similarly to chemical stimulation of sexual reproduction. The objectives of this study were to determine if non-sterol substances were also stimulatory to sexual reproduction in other species or isolates of the same species of *Phytophthora* and *Pythium*, and to test the ability of other *Phytophthora* species to synthesize substances needed for their sexual reproduction. Whether nutrient deprivation can also trigger the process of sexual reproduction in other pythiaceous fungi was also investigated.

Materials and Methods

Organisms and Chemicals

Fungi used in this study included three isolates of *Phytophthora boehmeria,* two isolates of *P. sojae*, and one isolate each of *P. cactorum*, *P medicaginis*, *Pythium aphanidermatum*, and *Py. myriotylum* (Table 1).

Soybean lecithin, monopalmitin, dipalmitin, and tripalmitin (purchased from Sigma) were all 99% pure. The lecithin in chloroform was evaporated to dryness and then dissolved in ether before use to prevent it from becoming mucilaginous.

Bioassay

The basal medium of Jee and Ko (1997) was used for maintenance of test organisms and for bioassay. It consisted of 0.1 g KNO_3, 0.2 g K_2HPO_4, 0.1 g $MgSO_4$, 0.1 g $CaCl_2$, 1 ml trace elements, 0.1 g L-asparagine, 0.05 g L-serine, and 4 g glucose in 1 l of distilled water. The trace element solution contained 200 mg FeEDTA, 10 mg $CuSO_4$, 10 mg $MnCl_2$, 10 mg Na_2MoO_4, 10 mg $Na_2B_4O_7$, and 20 mg $ZnSO_4$, and 100 mg thiamine hydrochloride in 100 ml distilled water. Highly purified SeaKem agarose (HGT-P Agarose, FMC, BioProducts, Rockland, ME, USA.), which contained no detectable nutrient contaminants (Ho and Ko, 1980; Ko, 1985), was used to solidify the basal medium at a concentration of 0.8% (w/v). The medium was adjusted to pH 6.2 with 0.5 N KOH. For each test organism, a piece (ca 3×3×3 mm) of culture grown on 10% V-8 agar (10% V-8 juice, 0.02% $CaCO_3$, and 2% agar) was placed in the center of a basal medium plate and incubated at 25°C in darkness. After the colony reached about 4 cm in diameter, the same size of culture was cut from the advancing margin and placed in the center of a plate containing fresh basal medium. The procedure was repeated three times before use to avoid possible contamination of nutrients from the original inoculum. Under these conditions, none of the test organisms formed oospores on the basal medium unless a stimulatory substance, such as lecithim, was added, indicating that stimulatory substances were not carried over from the inoculum.

Three basal medium blocks (10×10×5 mm) were evenly placed in a 6-cm Petri plate, and 20 µl of ether containing 500 µg of lecithin or ethyl acetate containing 100 to 500 µg of other substances was spread over the surface of a basal medium block. After evaporation of the organic solvent in a fume hood for 30 min, the basal medium blocks were each inoculated with a small cube (ca 2×2×2 mm) of a test organism. Inoculated blocks in Petri plates were incubated for 7 days at 25°C in darkness in a moist chamber for oospore production. To determine the number of oospores produced, each block was triturated with 5 ml of water in a mixer at 4,000 rpm for 1 min. Oospore concentration in the suspension was determined by counting the number of oospores in a 100 µl sample (Ko et al., 1973).

Table 1. List of isolates of *Phytophthora* and *Pythium* used.

Species and isolate	Host	Location
P. boehmeriae		
Ec-8	Cotton	China
Js-2	Cotton	China
Jc-20	Cotton	China
P. cactorum		
Ap-14	Apple	China
P. medicaginis		
44390	Alfalfa	Canada
P. sojae		
CN-1	Soybean	Canada
S317	Soybean	China
Py. aphanidermatum		
Py-4	Cucumber	China
Py. myriotylum		
357F	Pepper	Hawaii

Washing with NaCl Solution

The method developed by Jee and Ko (1997) was used. Ten mg of a test chemical dissolved in 50 ml of ether was vigorously shaken with 100 ml of 1% NaCl solution adjusted to pH 8 with 0.5 N KOH in a 500 ml separatory funnel for 1-2 min. After 2-3 h standing, the ether layer was collected and dried on a rotary evaporator. Ten ml of ether was added to the evaporation flask to dissolve the residue, and 10 g of anhydrous sodium sulphate was added to remove remaining water molecules. Ether containing the residue was transferred to a test tube and evaporated to dryness. The residue was redissolved in 1 ml of ethyl acetate for bioassay.

Thin-Layer Chromatography

Ten mg of the test chemical dissolved in 0.5 ml of ether was spotted on a TLC plate (10×20 cm, 60 A silica gel, layer 1 mm thick, Whatman adsorption plate, Fisher Scientific, Pittsburgh, PA, USA.) using 7 µl per spot (Jee and Ko, 1997). The loaded plate was developed with a mixture of hexane-ethyl ether-acetic acid (70:30:1), dried and visualized with iodine vapor to locate sample bands. After evaporation of iodine, the sample band was placed in a 25 ml centrifuge tube, and extracted with two aliquotes of 20 ml of ether. The combined ether solution was washed with 80 ml of 1% NaCl solution as described above.

Washed samples were dissolved in ethyl acetate for bioassay.

Florisil Column Chromatography

The method of Carroll (1961) was used to purify palmitic glycerides by Florisil column chromatography. Non-treated Florisil (30 g, 60-100 mesh; Fisher Scientific, Pittsburgh, PA, USA.) was packed in a column (20×30 cm) with 60 ml of hexane. Excess hexane was drained from the bottom before 200 mg of palmitin in 2 ml of hexane was loaded on top of the Florisil in the column. The Florisil column was washed with 50 ml of hexane and with 150 ml of 2% methanol in ether at the flow rate of 3 ml/min to remove non-target compounds. The palmitin was eluted by passing 150 ml of 4% acetic acid in ether through the column. The ether fraction was evaporated in the rotary evaporator, and the residue was dissolved in 10 ml of ethyl acetate for bioassay.

Effect of Nutrient Deprivation on Oospore Formation

A culture block (11×11×5 mm) obtained from a 7-day-old culture established on basal medium was placed in a 6-cm Petri plate containing 5 ml of 0.8% water agarose (SeaKem HGT-P agarose). After incubation for 7 days at 25°C in darkness in a moist chamber, oospores were counted directly under a microscope. Three plates were used for each test organism, and the experiment was done twice. One of the two experiments with similar results was presented.

Table 2. Oospore formation by species of *Phytophthora* and *Pythium* in basal agarose blocks (10×10×5 mm) each with 500 µg lecithin[a] added to the surface.

Species and isolate	No. oospores ± SD/block	
	Without lecithin	With lecithin
P. boehmeriae		
Ec-8	0	2311 ± 530
P. cactorum		
Ap-14	0	1961 ± 443
P. sojae		
S317	0	1606 ± 216
Py. aphanidermatum		
Py-4	0	1922 ± 533

[a]Lecithin was washed with NaCl solution before use.

Extraction of Stimulatory Substance from Mycelia

Two 6-cm plates of 14-day-old culture grown on basal agarose medium were triturated with 25 ml sterile distilled water in a mixer. One ml of culture suspension was added to a 250-ml flask containing 50 ml double strength liquid basal medium. After incubation for 14 days at 25°C in darkness, mycelia were harvested by filtration through filter paper, washed with 200 ml distilled water, and dried overnight at 60°C. Extraction was carried out following the method described previously (Jee and Ko, 1998). About 10 g dried mycelia was ground in a mortar and added to a 500-ml boiling flask containing 210 ml 95% (v/v) ethanol and 35 ml 50% (w/v) KOH. After gentle boiling under a reflux condenser for 2 h, the mixture was adjusted to 500 ml with deionized water. Non-saponifiables were extracted from the mixture by three aliquotes of 35 ml petroleum ether. The saponifiable fraction was obtained by adjusting the aqueous portion to pH 2 with 6 M HC1 and extracting the acidified solution with petroleum ether. Both non-saponifiable and saponifiable fractions were evaporated, and the residues were dissolved in 1 ml ethyl acetate for bioassay.

Results

After being washed with NaCl solution, lecithin was stimulatory to the oospore formation of *P. boehmeriae*, *P. cactorum, P. sojae*, and *Py. aphanidermatum* (Table 2). These four species of pythiaceous fungi were similar in responsiveness to the stimulation of lecithin. Among the three methods tested for purification of glycerides, FCC was most effective, followed by NaCl and TLC (Table 3). Without treatment, monopalmitin, dipalmitin, and tripalmitin with 99% purity were not effective in inducing sexual reproduction of *P. cactorum*. However, after purification by FCC all three glycerides of palmitic acid were stimulatory to oospore formation at concentrations of 200 µg and 500 µg/block (Table 3). Monoplamitin was most effective in inducing the sexual reproduction of *P. cactorum*, followed by dipalmitin and tripalmitin. After treatment with NaCl or TLC, these compounds were stimulatory to

Table 3. Effect of purification methods on the stimulatory effect of various glycerides of palmitic acid on oospore formation in *Phytophthora cactorum*.

Basal medium supplement	No. oospores ± SD/block							
	200 µg/block				500 µg/block			
	NT[a]	NaCl	TLC	FCC	NT	NaCl	TLC	FCC
Monopalmitin	0	0	0	1280 ± 147	0	40 ± 27	34 ± 27	3937 ± 318
Dipalmitin	0	0	0	137 ± 26	0	198 ± 23	425 ± 25	523 ± 120
Tripalmitin	0	0	0	98 ± 32	0	65 ± 18	162 ± 18	215 ± 42

[a]NT, no treatment; NaCl, washed with NaCl solution; TLC, thin layer chromatography; FCC, Florisil column chromatography.

Table 4. Stimulatory effect of various glycerides of palmitic acid purified by FCC on oospore formation by species of *Phytophthora* and *Pythium*.

Species and isolates	No. oospres ± SD/plate					
	200 µg/block			500 µg/blcok		
	Monoplamitin	Dipalmitin	Tripalmitin	Monopalmitin	Dipalmitin	Tripalmitin
P. boehmeriae						
Ec-8	0	0	0	1080 ± 253	122 ± 29	16 ± 5
Js-2	2673 ± 348	0	0	5140 ± 718	172 ± 16	210 ± 43
Jc-20	2178 ± 293	0	0	3488 ± 923	390 ± 47	145 ± 27
P. medicaginis						
44390	0	0	0	0	0	0
P. sojae						
S317	1532 ± 342	88 ± 30	132 ± 10	2835 ± 432	557 ± 102	490 ± 70
CN-1	0	0	0	0	0	0
Py. myriotylum						
357F	0	0	0	0	0	0

oospore formation only at 500 µg/block. These glycerides purified by FCC were, therefore, studied for their effect on other test organisms. The result showed that different species and even different isolates of the same species responded differently to stimulation by glycerides. The palmitic glycerides were stimulatory to oospore formation of *P. boehmeriae* and *P. sojae*, but not *P. medicaginis* or *Py. myriotylum* (Table 4). For *P. sojae*, although all these glycerides were stimulatory to oospore formation of isolate S317, none of them had any stimulatory effect on isolate CN-1. Among three isolates of *P. boehmeriae* tested, isolate Js-2 was most responsive to stimulation by all three glycerides followed by isolate Je-20 and isolate Ec-8. In general, monopalmitin was most effective in inducing sexual reproduction, followed by dipalmitin and tripalmitin (Table 4).

When *Py. aphanidermatum* growing on basal medium was subjected to nutrient deficiency by transfer to water agarose, the mycelia grew sparsely but steadily and reached the edge of the Petri plates and formed oospores in 7 days (Table 5). However, the growth of *P. boehmeriae, P. cactorum* and *P. sojae* was very weak and slow after being transferred to water agarose. The mycelia extended less than 5 mm away from the inocula after 2 months, and no oospores were produced.

Extracts from mycelia of each organism grown in liquid basal medium were tested for ability to stimulate its own sexual reproduction. The non-saponifiables, excluding saponifiables, extracted from the mycelia of *P. boehmeriae, P. cactorum* and *P. sojae*, were stimulatory to its own oospore formation (Table 6). *Phytophthora boehmeriae* produced the largest amount of stimulatory substances followed by *P. cactorum* and *P. sojae*.

Discussion

Lecithin was known to be stimulatory to the sexual reproduction of only *P. cactorum*, *P. parasitica*, and *Py. aphanidermation* prior to the present study (Ko, 1985; Jee et al., 1997). In this study, lecithin purified by washing with NaCl was also found to be stimulatory to *P. boehmeriae* and *P. sojae*, and isolates of *P. cactorum* and *Py. aphanidermatum* different from those tested previously. These results suggest that lecithin has a broad spectrum of stimulatory effect on the sexual reproduction of pythiaceous fungi. The only tested organism not responsive to lecithin was *Pythium vexans* (Ko, 1985). Since commercial lecithin is known to contain a substance inhibitory to oospore formation (Jee et al., 1997), and the lecithin used during that time was not pre-washed, oospore

Table 5. Oospores formation by species of *Phytophthora* and *Pythium* following transfer from basal agarose medium to water agarose.

Species and isolates	No. oospores ± SD/plate
P. boehmeriae	
Ec-8	0
P. cactorum	
Ap-14	0
P. sojae	
S317	0
Py. aphanidermatum	
Py-4	248 ± 74

Table 6. Effect of substances extracted from mycelia of *Phytophthora* species on oospore formation by the same species.

Species and isolates	No. oospores ± SD/100 µg sample	
	Non-saponifiable	Saponifiable
P. boehmeriae		
Ec-8	7640 ± 978	0
P. cactorum		
Ap-14	5420 ± 573	0
P. sojae		
S317	3577 ± 297	0

formation of *Py. vexans* was probably suppressed by the inhibitor in the lecithin rather than being non-responsive to lecithins per se.

In this study, commercial palmitic glycerides of 99% purity were found to contain a substance inhibitory to oospore formation. Purification with FCC was more effective in removing the inhibitory substance than purification with TLC or washing with NaCl solution. After purification with FCC, palmitic glycerides were found to be stimulatory to sexual reproduction of *Phytophthora* for the first time. The stimulatory effect of these compounds appeared to be very specific. They were stimulatory to oospore formation of *P. boehmeriae* and *P. sojae* but not *P. medicaginis*. Moreover, these compounds were stimulatory to isolate S317 of *P. sojae* but not isolate CN-1 of the same species.

Pythium aphanidermatum cultured on basal medium grew sparsely but steadily and produced oospores after being transferred to nutrient-free water agarose. This is the first time a species of *Pythium* was shown to have the ability to produce oospores without the presence of any stimulatory substances. However, growth of *P. boehmeriae*, *P. cactorum*, and *P. sojae* was extremely slow and limited, and no oospores were produced after transfer from basal medium to water agarose. The amount of basal medium used in this study was apparently not enough for mycelia of *Phytophthora* to obtain nutrients sufficient for continuation of growth and production of oospores after being transferred to nutrient-free medium. There was a direct relationship between the amount of basal medium used for mycelial growth and the amount of oospores produced after being transferred to water agarose (Jee and Ko, 1998). In this study, extracts from mycelia of *P. boehmeriae, P. cactorum*, and *P. sojae* grown in liquid basal medium were stimulatory to their own sexual reproduction. Our results support the hypothesis of Jee and Ko (1998) that pythiaceous fungi can synthesize substances needed for sexual reproduction but require a stress factor to trigger the process.

Literature Cited

Barnett, J.H. 1976. Fundamental of Mycology. Crane Russak & Co., New York.

Bidwell, R.G.S. 1974. Plant Physiology. Macmillan Publishing Co, New York.

Carroll, K.K. 1961. Separation of lipid classes by chromatograpy on florisil. J. Lipid Res. **2:** 135-141.

Elliott, C.G. 1983. Physiology of sexual reproduction in *Phytophthora*. *In* D.C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao (eds.), *Phytophthora*, Its Biology, Taxonomy, Mycology, and Pathology, American Phytopathological Society, St. Paul, Minnesota, pp. 71-80.

Elliot, C.G., M.E. Hendrie, B.A. Knights, and W. Parker. 1964. A sterol growth factor requirement in a fungus. Nature **203:** 427-428.

Erwin, D.C. and O.K. Ribeiro. 1996. *Phytophthora* Diseases Woldwide. APS Press, St. Paul Minnesota.

Harnish, W.H., L.A. Berg, and V.G. Lilly. 1964. Factors in lima bean and hemp seed required for oospore formation by species of *Phytophthora*. Phytopathology **54:** 895.

Haskins, R.H., A.P. Tulloch, and R.G. Micetich. 1964. Steroids and the stimulation of sexual reproduction of a species of *Pythium*. Can J. Microbiol. **10:** 187-195.

Hendrix, J.W. 1964. Sterol induction of reproduction and stimulation of growth of *Pythium* and *Phytophthora*. Science **144:** 1028-1029.

Hendrix, J.W. 1970. Sterols in growth and reproduction of fungi. Annu. Rev. Phytopathol. **8:** 111-130.

Ho, W.H. and W.H. Ko. 1980. Agarose medium for bioassay of antimicrobial substances. Phytopathology **70:** 764-766.

Jee, H.J. and W.H. Ko. 1997. Stimulation of sexual reproduction of *Phytophthora cactorum* and *P. parasitica* by fatty acids and related compounds. Mycol. Res. **101:** 1140-1144.

Jee, H.J. and W.H. Ko. 1998. *Phytophthora cactorum* can synthesize substances need for sexual reproduction but requires a stress factor to trigger the process. Microbiology **144:** 1071-1075.

Jee, H.J., C.S. Tang, and W.H. Ko. 1997. Stimulation of sexual reproduction in *Phytophthora cactorum* by phospholipids is not due to sterol contamination. Microbiology **143:** 1631-1638.

Ko, W.H. 1985. Stimulation of sexual reproduction of *Phytophthora cactorum* by phospholipids. J. Gen. Microbiol. **131:** 2591-2594.

Ko, W.H. 1986. Sexual reproduction of *Pythium aphanidermatum*: stimulation by phospholipids. Phytopathology **76:** 1159-1160.

Ko, W.H. 1988. Hormonal heterothallism and homothallism in *Phytophthora*. Annu. Rev. Phytopathol. **26:** 57-73.

Ko, W.H. 1998. Chemical stimulation of sexual reproduction in *Phytophthora* and *Pythium*. Bot. Bull. Acad. Sin. **39:** 81-86.

Ko, W.H., L.L. Chase, and R.K Kunimoto. 1973. A microsyringe method for determining concentration of fungal propagules. Phytopathology **63:** 1206-1207.

Ko, W.H. and W.C. Ho. 1983. Reassessment of apparent sterol requirements for sexual reproduction in *Phytophthora*. Ann. Phytopathol. Soc. Japan **49:** 316-321.

Leal, J.A., J. Friend, and P. Holiday. 1964. A factor controlling sexual reproduction in *Phytophthora*. Nature **203:** 545-546.

Naggle, G.R. and G.J. Fritz. 1976. Introductory Plant Physiology. Prentice-Hall, Englewood Cliffs.

Nes, W.D. 1987. Biosynthesis and requirement for sterols in the growth and reproduction of Oomycetes. *In* G. Fuller and W.D. Nes (eds.), Ecology and Metabolism of Plant Lipids, American Chemical Society, Washington D.C., pp. 304-628.

Smith, J.A. and D.R. Berry. 1974. An Introduction to Biochemistry of Fungal Development. Academic Press. New York.

Van der Plants-Niterick, A.J. 1981. Monograph of the genus *Pythium*. Stud. Mycol. **21:** 1-242.

Webster, J. 1980. Introduction to Fungi. Cambridge University Press, London.

菌株源對化學誘導 *Phytophthora* 和 *Pythium* 之有性生殖的影響

吳 浩[1] 鄭小波[1] 柯文雄[2]

[1]南京農業大學植物保護系
[2]夏威夷大學植物與環境科學系

卵磷脂經氯化鈉水溶液清洗可以誘導 *Phytophthora boehmeriae, P. sojae, P. catorum* 和 *Pythium aphanidermatum* 卵孢子形成。一種商品化甘油脂具有 99% 純度之棕櫚酸含有抑制卵孢子形成物質。利用矽酸鎂管柱分析純化去除抑制物質比薄層分析或氯化鈉清洗之效果好。經純化後，單棕櫚酸精、雙棕櫚酸精和三棕櫚酸精均具有誘導 *Phytophthora* 之有性生殖。這些化合物可以誘導 *P. boehmeriae* 和 *P. sojae* 形成卵孢子，但對 *P. medicaginis* 沒有效果，然而對 *P. sojae* 不同菌株間也有差異，如對菌株 S317 有誘導作用，但對菌株 CN-1 則沒有作用。*Py. aphanidermatum* 培養於基本培養基上，其菌絲生長稀疏，但移植於沒有養分之純水瓊脂培養基可以穩定形成卵孢子。將培養於液體基本培養基之 *P. boehmeriae, P. cactorum* 和 *P. sojae* 菌絲萃取可以誘導自身菌種形成有性生殖。本研究結果支持下述之假說：pythiaceous 真菌在環境逆壓下，可以啟動合成誘導有性生殖之物質。

關鍵詞：卵磷脂；甘油脂；有性生殖；*Phytophthora* spp.；*Pythium* spp.。