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Phytophthora species associated with strawberry fruit rot in Taiwan

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Abstract. Three species of the genus *Phytophthora* were found to be associated with strawberry fruit rot. They were *P. cactorum, P. citrophthora,* and *P. citricola. Phytophthora citricola* was the first time reported being isolated from strawberry fruit rot and has not been documented anywhere before. We suggested that *P. citrophthora* and *P. citricola* which associated with strawberry fruit rot were originated from nearby citrus orchard plantations. A *Phytophthora* sp. designated as F-798 isolated from brownish stem tissue of stunting strawberry was identical with a high temperature isolate of *P. megasperma* which caused alfalfa root disease in California. Based on morphology of sporangia, pattern of repeatedly internal proliferation of renew sporangia, oospore formation in strawberry brownish stem tissue, and formation of no oospores in laboratory media we inclined to suggest that isolate F-798 belongs to *P. fragariae* but with high optmal temperature for mycelial growth, ranging from 25° to 35°C and best at 30°C. No spread out of this fungus has been detected based on isolation tests. Isolate F-794, another *Phytophthora* sp. found to be associated with strawberry, was also isolated from stunting strawberry brownish stem tissue, produced non-papillate sporangia and chlamdospore-like bodies. Both isolates F-798 and F-794 produced no oospore singly, however, isolate F-798 was determined as A2 mating type.

Key words: *Phytophthora cactorum*; *P. citricola*; *P. citrophthora*; High temperature *P. fragariae*; Strawberry fruit rot.

Introduction

Strawberry (*Fragaria chilensis* Ehrh) has been widely cultivated in Taiwan for the past ten or more years mainly in Dah-hu, Miawli county, however, it has extended to both south and north parts of the west coast of island for the past few years. Practically strawberry has been rotated with rice annually and growing season starts from December to May of the next years. It was of our original interest to investigate whether red core disease of strawberry had been brought in together with seed runners either from the United States or Japan. Red core disease of strawberry was caused by *Phytophthora fragariae*, a species of *Phytophthora* erected by Hickman in 1940 in Scotland (Hickman, 1940). Red core disease of strawberry has been reported in most of the strawberry growing regions (Montgomerie, 1977).

Phytophthora fruit rot of strawberry, instead of red core, has been found to be the most devastating disease encountered in Taiwan especially when excessive rain occurred. In 1983 there was an unusually long period of continued rain. Rain had continued from Feburary to April islandwide and had caused a severe fruit rot in the fields as

well as after harvest all over the island. A survey on the species of the genus *Phytophthora* associated with strawberry fruit rot was conducted. This paper reports the results of our study on the species of *Phytophthora* on strawberry.

Material and Methods

Isolation of Causal Fungi of Strawberry Fruit Rot

Rotten strawberry were collected from fields at the following locations: Shin-dien (Taipei county), Kuan-hsi (Shin-tsu county), Dah-hu (Miawli county), Dah-chia (Taichung county), Hou-li (Taichung county), Shen-kang (Taichung county), Kuo-hsing (Nantou county) and Doou-nan (Yuinlin county). A piece (0.3 cm^3) of rotten fruit tissue cut from each diseased fruit was placed in a 6 or 9 cm Petri dish and dripped with tap water overnight to eliminate contaminated bacteria as much as possible and to induce sporulation. Hyphae and sporangia usually appeared on the tissue blocks 10 h after dripping with water. The tissue blocks were then washed twice with sterile water. Excessive water was blotted with layers of sterile tissue paper and placed on selective medium (Ko *et al.*, 1978). Forty eight hours after incubation at room temperature characteristic *Phytophthora* hyphae emerged and developed into a colony. Hyphal tips were cut and subcultured, purified and identified to the species. Identification was based on systems established by Tucker (1931), Waterhouse (1965), and Newhook *et al.* (1978).

Isolation Phytophthora spp. from Diseased Plant Underground Parts

Fungus isolation was conducted by cutting a piece of tissue from the inner part of diseased underground stem which was first surface sterilized with 75% ethanol and placed on a selective medium. The plates were incubated at 20°, 25°, and 30°C for 3 days and then examined the hyphae emerged from isolaing tissue through the bottom of plate under a dissecting microscope. Hyphal tip subcultures were made onto V-8 juice agar surface inside a van Tieghem cell which was placed in dish before agar solidified. Hyphae grew through the bottom end of van Teighem cell and emerged on the agar surface outside of van Tieghem cell. After three repeats pure cultures were ensured. All isolation cultures were grown on V-8 juice agar slants and incubated at 15° to 20°C under darkness.

Mycelial Growth of a Phytophthora Isolate F-798 in Relation to Temperature

To determine the range of temperature optimal for mycelial growth of the isolate F-798 of a *Phytophthora* sp. a mycelial agar block of 3 mm in diameter cut from the margin of a active growing colony was transferred to the center of a 9 cm Petri plate containing 15 ml of 10% V-8 juice agar. Three plates were tested for each temperature. Temperatures were 15°, 20°, 25°, 30°, 35°C and 37°C. Four days after incubation growth rate of colonies were measured. All tests were repeated once.

Preparation of Solutions to Induce Sporangium Formation of Phytophthora sp. Isolate F-798

Besides tap water three solutions were used to induce sporangium formation. They were soil extract solution, Petri solution (Booth, 1971) and Chen-Zentmyer mineral solution (Chen and Zentmyer, 1970). Soil extract solution was prepared by mixed 10 g rice paddy field soil with 500 ml tap water and filtered through a layer of filter paper to obtain a clean soil extract solution. All solutions were stored in 5°C before use.

Results and Discussion

***Phytophthora** spp. Associated with Strawberry Fruit Rot*

Phytophthora cactorum, P. citricola, P. citrophthora and a *P. fragariae* like species desig-

Table 1. *Species of the genus* ***Phytophthora*** *associated with strawqerry fruit rot at different locations in Taiwan*

Location	*P. cactorum*	*P. citrphtohora*	*P. citricola*	*F-798*
Shih-dien	+	+	+	—
Kuan-hsi	—	+	—	—
Kah-hu	+	+	—	—
Dah-chia	+	—	—	+
Hou-li	—	+	—	—
Shih-kaon	+	+	—	—
Kuo-hsing	+	+	+	—
Doou-nan	+	—	—	—

+: presence of the fungus indicated.
—: absence of the fungus indicated.

nated as isolate F-798 were found to be associated with strawberry fruit rot (Table 1). *P. cactorum* was the most widely distributed species found to be associated with strawberry fruit rot. *P. citrophthora* was first time reported to incite strawberry fruit rot by Kao and Leu in 1977 at Dah-hu. Present investigation demonstrated that this fungus also existed in other 6 strawberry fields. *P. citricola* was the first time reported to be associated with this disease and only isolated from rotten fruits collected at Shin-dien and Kuo-hsing. *P. cactorum* was the dominated species based on our own isolation survey for the last few years, it had been isolated from all investigated locations except Kuan-hsi. *P. citrophthora* was the dominated species at Shin-dien strawberry field although *P. citricola* was also frequently isolated. The source of these two species at Shin-dien fields was traced and found to be coming from the nearby citrus orchards up on the low hill. Soil samples collected from those citrus orchards and fungus isolation resulted *P. citrophthora*. Based on our observations and isolations we believe that *P. citrophthora* and *P. citricola* which associated with strawberry fruit rot were originally come from nearby citrus orchards. Isolate F-798 was isolated only once from rotten fruit collected at Dah-chia.

Phytophthora spp. Isolated from Underground Part of Stunting Strawberry

An isolate of *Phytophthora* sp. designated as F-798 was obtained from stunting strawberry brownish stem tissue which was collected in June and July of 1978 from the same field at Dah-hu. However, in 1979 none of the same fungus was isolated from brownish stem tissue of stunting strawberry collected at the same and nearby fields where isolate F-798 was obtained in the previous year. Instead another *Phytophthora* sp. designated as F-794, was isolated. Not until August 1979 isolate F-798 was again isolated from brownish stem tissue of stunting strawberry collected at a small plot which was apparently left to serve as stock to propagate runners for planting in the next seaon. Isolate F-794 was also once isolated from brown rot of passion fruit collected on the ground nearby field where isolate F-794 was previously isolated. Based on above-mentioned facts we suggest that isolates F-798 and F-794 of *Phytophthora* spp. are still restricted, for unknown cause, in sporadical areas and not spreaded out except once isolate F-798 being isolated from a rotten fruit collected at Dah-chia in 1983. It has already been domonstrated that stunting of strawberry in Dah-hu was caused by a race of

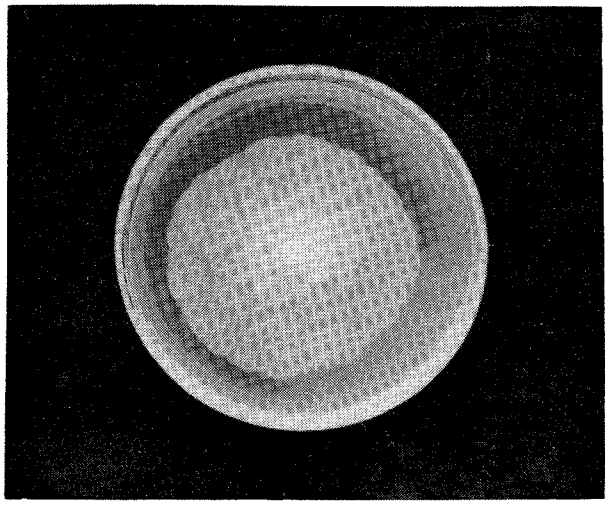

Fig. 1. Colony of isolate F-798 of *Phytophthora* sp.

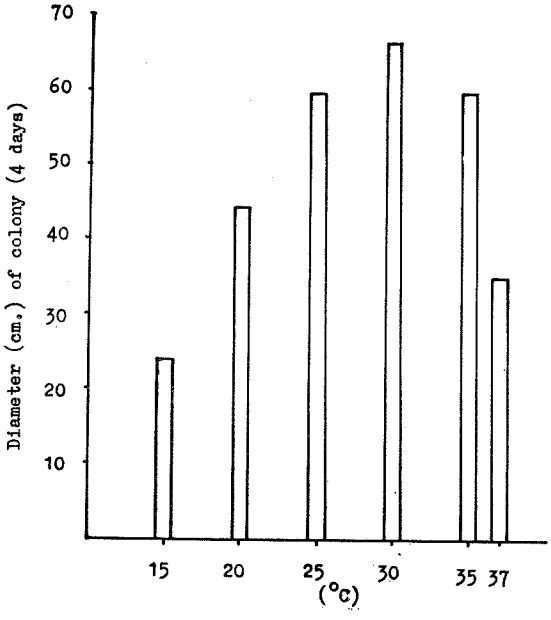

Fig. 2. Temperature in relation to mycelial growth of *Phytophthora* isolate F-798.

Pseudomonas solanacearum (Hsu, 1984).

Brief account of morphology and behavior of life history of isolates F-798 and 794 of *Phytophthora* spp. are given as follows:

Isolate F-798. This fungus grew well on V-8 juice agar and developed into a chrysanthemum type colony with thin aerial mycelia (Fig. 1). Hyphae was fairly uniform without profuse swelling but formed chlamydospore-like bodies, terminate, frequently in group and 30 to 36 μ in diameter. Isolate F-798 grew well ranging from 25° to 35°C but relatively poor at 15°C (Fig. 2). Sporangia usually ovoid to obpyriform, non-papillate, 39-53×28-34 μ (Fig. 3, a, b). Sporangia formed only when mycelial agar blocks were submerged in mineral solutions, such as Petri-solution and soil extract solution but no sporangia were observed on solid agar media. Sporangia produced best at 25°C to 28°C. Sporangium differentiated and produced zoospores, and released zoospores from sporangium immediately after sporangium mature. Under favorable conditions sporangia began to appear 3 to 5 h after mycelial mat submerged in soil extract solution incubated at 25°C under illumination. A simple and efficient method to produce sporangia and zoospores of this fungus and other non-papillate heterothallic species of the genus *Phytophthora* which we have obtained here in Taiwan is briefly described: A piece of mycelial agar block (0.4 cm in diameter) cut from actively growing colony margin was inoculated in 10 ml of 10% V-8 juice solution in a 6 cm dish and incubated at 25°C for 48 h. When mycelial mat reached about 2 to 2.5 cm in diameter, the culture solution was pipeted out and mycelial mat was rinsed twice with sterile distilled water, and then added 5 ml soil extract solution into dish. Twelve to 24 h after incubation at 25°C under illumination abundant sporangia were usually obtained. Tests also showed that soil extract solution was the best for inducing sporangium formation (Fig. 4). We also demonatrated that culture solution in which fungus was grown also affected the potential for sporangium formation. Mycelial mats grown in V-8 juice (10%) was better than 10% hamp seed (W/V), 10% carrot (W/V) and 10% cucumber (W/V) solution for sporangium formation (Fig. 5). Isolate F-798 produced no oospores in laboratory media so far had been tried except once produced in a clump of

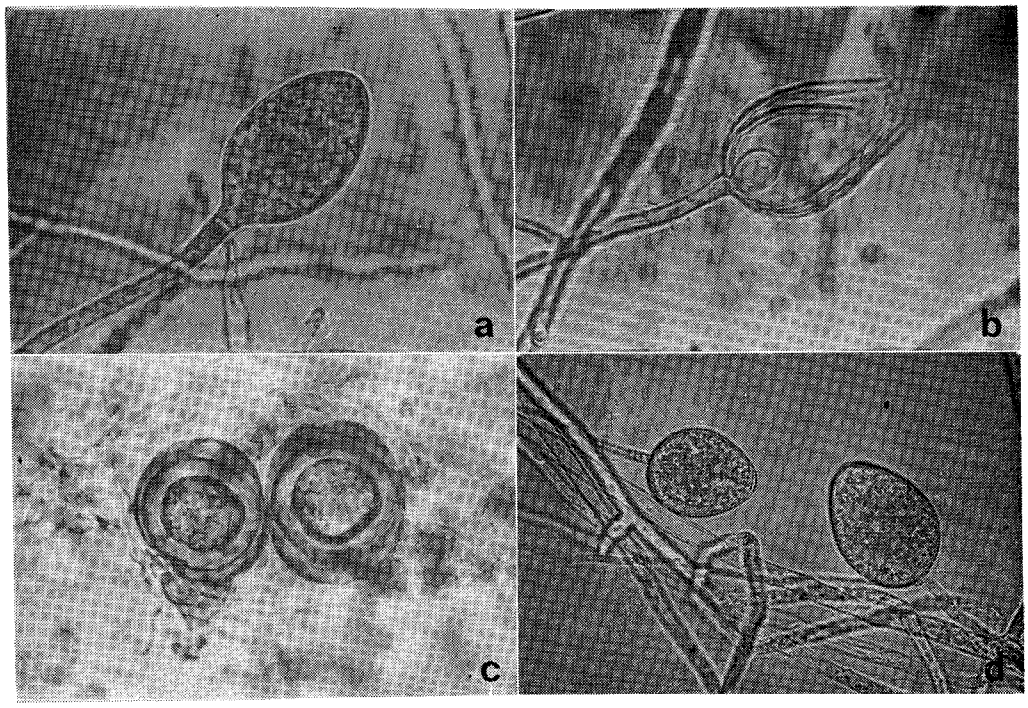

Fig. 3. Sporangium (a, b) and oospores (c) of isolate F-798 and sporangium (d) of isolate F-794 of *Phytophthora* spp. (×490)

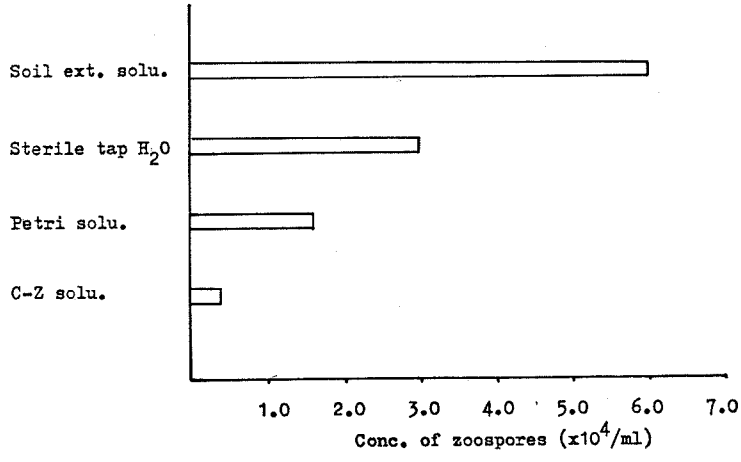

Fig. 4. Mycelial mats of isolate F-798 of *Phytophthora* sp. formed in 10% V-8 juice treated with different mineral solutions in relation to zoospore production.

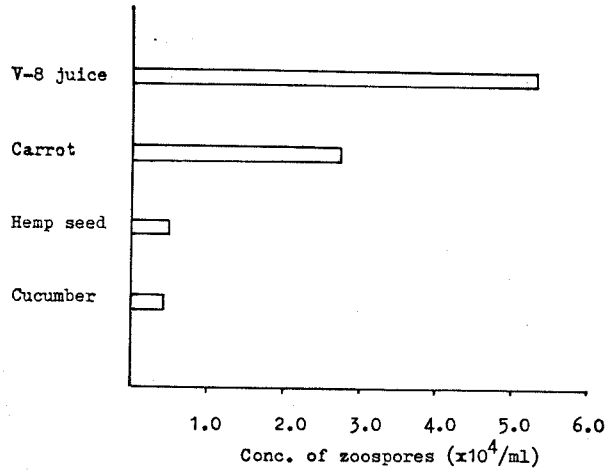

Fig. 5. Number of zoospores produced on mycelial mats of isolate F-798 of *Phytophthora* sp. grown in different liquid media and then treated with soil extract solution.

aggregate on oat meal agar, however, no repeat could be made. This fungus, nevertheless, induced isolate P731 (an A2 mating type) of *P. parasitica* and CO-1 of *P. colocasiae* (an A2 mating type) to produce oospores. Oospores were also observed in brownish stem tissue from there isolate F-798 was isolated. Oospores were thick-walled but no intact antheridia were found in our materials (Fig. 3c). Oogonium, 30-42 μ; oospores, 23-31 μ.

Ribeiro *et al.* (1977) isolated a high-temperature *Phytophthora* sp. from alfalfa which they suggested as a variant of *P. megasperma* based on the morphology of sporangia and hyphal swellings. Our isolate is very close to the fungus reported by Ribeiro *et al.* in culture characters and sporangium size and shape as well as the pattern of internal proliferation of new sporangium. Isolate F-798 released zoospores at 30°C as well as at 20° and 25°C. Sporangia differentiated and released zoospores immediately after maturation. No chilling was required to enhance indirect germination of sporangia. Isolate F-798 also produced oospores in diseased stem tissue, however, no oospores in diseased alfalfa tissue were found by Ribeiro *et al.* in their isolate. According to the literature available *P. megasperma* produced oospores readily in laboratory media (Newhook *et al.*, 1978) whereas *P. fragariae* was not easily to produce sexual organs at the same conditions but produced oospores in diseased tissue. Mass (1972) reported that some of California isolates of *P. fragariae* have rather high optimal temperature, i.e., 25° to 30°C instead of 18° to 20°C for mycelial growth. We believe that isolate F-798 is a fungus very close to *P. fragariae* or even a variant of *P. fragariae*.

Isolate F-794. This fungus was isolated from brownish stem tissue of stunting strawberry collected at Dah-hu in April 1979. It was also isolated from fallen passion fruit which showed brown rot patch. This fungus grew well on V-8 juice agar, few aerial mycelia. Sporangia broad ellipsoid, non-papillate (Fig. 3d), not caducous, 36-52×22-34 μ. New sporangium formation was either internal proliferation or sympodial type proliferation. Same as isolate F-798 no sporangia produced on solid agar media. Sporangium formed abundantly after submerged in soil extract solution or other minal solutions. This fungus also has rather high optimal temperature for mycelial growth. At 35°C it still grew fairly good. No sexual structures have been found.

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臺灣草莓果腐之關係疫病菌

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草莓果腐是目前臺灣草莓病害之一，尤其是綿雨不斷的時節，草莓果腐是因爲疫病菌感染所引起，主要者爲 *Phytophthora cactorum*，後又發現 *P. citrophthora* 也和草莓果腐有關連。本報告第一次發現 *P. citricola* 也和草莓果腐有關連。*P. citrophthora* 和 *P. citricola* 是柑桔褐腐和踏腐的病原菌，從新店廣興里草莓果腐和附近山丘桔園的地緣關係，從桔園土壤中的確分離到 *P. citrophthora* 和 *P. citricola* 初步證實引起草莓園草莓果腐的此二病原菌，很可能是從桔園土壤經雨水沖洗下來，經渠道擴散到草莓園引起草莓果腐，另有一株首次在1978從大湖的矮化草莓株褐化地下部分離到的疫病菌株，代號暫爲 F-798，後來在大甲草莓園果腐也分離過一次。這株菌和引起草莓紅柱心的疫病菌 *P. fragariae* 在形態上，包括孢囊形狀，孢囊的形成方式以及在病組織形成有性器官都非常類似，但 F-798 之生長適溫在 25～35°C 之間，而溫帶產 *P. fragariae* 的在 20°C 左右，另 F-798 菌株和最近在加州發現的一株引起苜蓿根部病害的好高溫 *P. megasperma* 菌株在形態上及菌絲生長溫度須求幾乎完全一樣，*P. megasperma* 極易在實驗用之培養基中形成有性器官，但此加州產之好高溫之 *P. megasperma* 則否。有性器官在病變組織容易找到，但在實驗用培養基上，只有在燕麥煎液培養基上配對培養產生過一次，後未能再重複，本菌株的確更接近 *P. fragariae*。