Botanical Studies (2006) 47: 273-277.

The possible origin and relation of *Phytophthora katsurae* and *P. heveae*, discovered in a protected natural forest in Taiwan

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(Received April 22, 2005; Accepted January 18, 2006)

ABSTRACT. When a total of 531 soil samples collected from 1976 to 2000 from various locations distributed in every county on the island of Taiwan was assayed, sixteen isolates of *Phytophthora katsurae* and nine isolates of *P. heveae* were recovered from three and four counties, respectively. *Phytophthora katsurae* and *P. heveae* were isolated from a protected natural forest located atop a hill at Lenhuachih without higher land within the range of vision, suggesting that both species are indigenous to Taiwan. *Phytophthora katsurae* is distinguished from the similar *P. heveae* by its verrucose oogonial wall. The number of protrusions produced by the Taiwanese isolates of *P. katsurae* varied greatly ranging from 2 to 23 per oogonium, indicating the unsteadiness of this taxonomical characteristic. Oogonia produced by isolates of *P. katsurae* obtained from Hawaii contained very few protrusions. Moreover, most oogonia produced by two of these isolates did not have any protrusions and were indistinguishable from those produced by *P. heveae*. Results suggest the development of *P. heveae* directly from *P. katsurae* by loss of oogonial protrusions. The high level of ITS sequence similarity between *P. heveae* and *P. katsurae* in comparison with their relationships to other *Phytophthora* species tested also support the possibility of the recent development of one species from the other species.

Keywords: *Phytophthora heveae*; *Phytophthora katsurae*; Oogonial protrusion; Indigenous.

INTRODUCTION

Phytophthora katsurae Ko and Chang (1979) closely resembles *P. heveae* Thompson in morphological characteristics with the exception of its verrucose oogonial wall (Ho et al., 1995; Stamps et al., 1990). Both species characteristically produce abundant oogonia with a funnel-shaped base and small amphigynous antheridia in a single culture (Ho et al., 1995). Sporangia of both species are papillate with hemispherical apical thickenings, and are non-deciduous in water (Ho et al., 1995). A high level of isozyme similarity was observed among the isolates of *P. katsurae* and *P. heveae* (Oudemans and Coffey, 1991). Cooke et al., (2000) showed that, based on ITS sequences of genomic rDNA, the 47 *Phytophthora* taxa examined are divisible into eight clades. *Phytophthora katsurae* and *P. heveae* are closely related sister taxa in a discrete clade. Recent phylogenetic analysis of *Phytophthora* species based on nuclear gene sequence and the combination of nuclear and mitochondrial gene sequences also showed a strong support for the close relationship of *P. katsurae* and *P. heveae* (Kroon et al., 2004).

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Geographic distribution and host range of *P. katsurae* are limited. Its known hosts include only chestnut (Katsura, 1976), coconut (Uchida et al., 1992), and cocoa (Liyanage and Wheeler, 1989). The organism is distributed in Japan, Taiwan, Hawaii, Ivory Coast, Australia, and Papua New Guinea (Stamps, 1985). Recently, it was reported from China for the first time. The organism was isolated from soil on Hainan Island in southern China (Ho et al, 2005).

Phytophthora heveae has slightly wider geographic distribution and host range than *P. katsurae.* The organism causes diseases on Kauri pine, Brazilian nut, guava, rhododendron, and cocoa (Erwin and Ribeiro, 1996). It has been reported from Malaysia, New Zealand, New Guinea, Brazil, Ivory Coast, Australia, India, Guatemala, and the United States (Erwin and Ribeiro, 1996). The organism has also been recovered from soil from Taiwan (Ho et al., 1995) and China (Zhang et al., 1995; Ho et al., 2005).

During our survey of the distribution of *P. katsurae* and *P. heveae* in Taiwan, both organisms were recovered from a protected natural forest in central Taiwan. The possible origin of these two species of *Phytophthora* and their possible evolutionary relationship were, therefore, investigated.

MATERIALS AND METHODS

Collection of soil samples

Soil samples collected from various locations were taken from a depth of 0 to 10 cm after the surface litter was cleared. Each location represented a different vegetation, soil type, or elevation, and the sample about 1 to 2 kg was a composite of three subsamples taken within a circle of approximately 1 m diameter. From 1976 to 2000, a total of 531 soil samples was collected intermittently from locations in every county on the island of Taiwan.

Description of the protected natural forest

The protected natural forest at Lenhuachih of Nan Tow County in central Taiwan (Figure 1) is under administration of the Taiwan Forestry Research Institute. It is a broad leaf forest with diverse tree species, a number of which belong to Lauraceae. The forest sits atop a hill without any higher land within the range of vision.

Isolation of organisms

Soils were assayed for the presence of *P. katsurae* and *P. heveae* within one week of collection by baiting with lupine radical (Chee and Newhook, 1965), citrus leaf discs (Grimem and Alesander, 1973), or leaf discs of camellia or azalea (Zhou et al., 1992), depending on availability of baiting materials at the time of soil collection. About 50 g of soil from each sample was mixed with 150 ml distilled water in a 400-ml plastic beaker. Each beaker contained five lupine radicals or leaf discs as baits. After 3-4 days at 24°C, baits were blotted dry and placed on a selective medium for pythiaceous organisms (Ko et al., 1978). The medium consisted of 5% V-8 juice, 0.02% $CaCO_3$, and 2% agar supplemented with 100 ppm ampicillin, 50 ppm nystatin, and 10 ppm pentachloronitrobenzene after autoclaving. Plates were incubated on the bench at 24°C. Two plates were used for each soil sample. *Phytophthora katsurae* was identified by its verrucose oogonia with a funnel-shaped base and a small amphigynous antheridium (Ko and Chang, 1979), while *P. heveae* was identified by its smooth oogonia with a funnel-shaped base and a small amphigynous antheridium (Ho et al., 1995).

Morphological observation

Since the structure of sporangia of *P. katsurae* and *P. heveae* are simple and similar (Ho et al., 1995), only sexual structures were included in this study. Isolates of *P. katsurae* and *P. heveae* were grown on a medium consisting of 10% V-8 juice, 0.02% $CaCO_3$ and 2% agar. After incubation at 24°C in darkness for 10 days, 20 oogonia from each isolate were randomly selected for determining the number of protrusion on each oogonium under a microscope . The experiments were done twice.

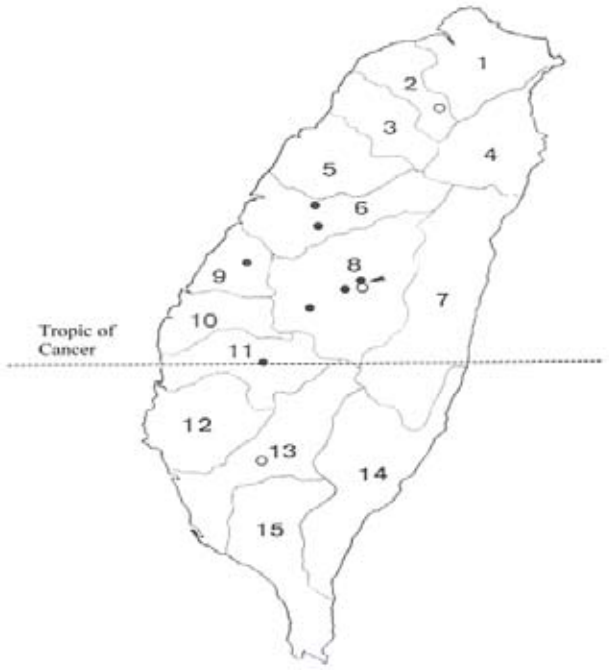

Figure 1. Distribution of *Phytophthora heveae* (black dots) and *P. katsurae* (open circles) in Taiwan, which is 380 km long from north to south and with an east-west maximum width of 140 km. Arrow points to the location of Lenhuachih from where both *P. heveae* and *P. katsurae* were isolated from a protected natural forest. Each number represents a county: 1, Taipei; 2, Tao Yuan; 3, Hsin Chu; 4, I Lan; 5, Miao Li; 6, Taichung; 7, Hua Len; 8, Nan Tow; 9, Chang Hua; 10, Yun Lin; 11, Chia Yi; 12, Tainan; 13, Kaohsiung; 14, Taitung; 15, Pintung.

Table 1. Species and isolates of *Phytophthora* used in sequence similarity analysis and GenBank accession numbers for ITS sequences[a].

Species and isolate	Associated habitat	Location	Accession number
P. heveae IMI180616	Hevea brasiliensis	Malaysia	AF266770
P. humicola IMI302303	Citurs orchard soil	Taiwan	AF266792
P. katsurae IMI360596	Cocos nucifera	Ivory Coast	AF266771
P. palmivora UQ1294	Theobroma cacao	Papua New Guinea	AF266780

[a]From data published by Cooke et al. (2000).

Sequence similarity analysis

The ITS sequences of genomic rDNA of *Phytophthora* species used in this study were published by Cooke et al. (2000). Their sequences were retrieved from GenBank for similarity analysis (Table 1). The nucleotide alignments were carried out using the optimal alignment method of the DNAMAN software (Version 4.0, Lynnon BioSoft, Quebec, Canada).

RESULTS AND DISCUSSION

Nine isolates of *P. heveae* were recovered from soil samples collected from farms cultivated with pineapple, citrus, peach, or longan, and a protected natural forest at Lenhuachih in Nan Tow County. *Phytophthora heveae* was also found in the counties of Taichung, Chang Hua and Chia Yi (Figure 1). A total of 16 isolates of *P. katsurae* was recovered from natural forests located at Sutsi in Tao Yuan County, Lenhuachih in Nan Tow County, and Tuona in Kaohsiung County (Figure 1).

One isolate of *P. katsurae* obtained in 1976 was lost. The number of protrusions on each oogonium produced by isolates of *P. katsurae* varied greatly, ranging from 2 to 23 per oogonium. The average number of protrusions produced by each isolate also varied from 10 to 17 (Table 2). None of the nine isolates of *P. heveae* obtained produced oogonia with protrusions.

Both *P. katsurae* and *P. heveae* were isolated from the protected forest at Lenhuachih in central Taiwan (Figure 1). The forest sits atop a hill, and no higher land is within the visible range. Consequently, it is not possible for these organisms to be transported to the forest by rain water or streams from nearest habitation. It is also very unlikely that these organisms were carried to the forest on the boots of humans or the hooves of wild animals (Kliejunas and Ko, 1976) because of the rarity *P. katsurae* and *P. heveae* in Taiwan. Our study, therefore, suggests that both organisms are indigenous to Taiwan. Failure to isolate *Phytophthora cinnamomi* Rands from undisturbed localities in the Americas has been used by Zentmyer (1979) as evidence that it is not indigenous to the Americas.

Phytophthora heveae, with a wider host range, has been isolated from cultivated land and also from natural forests in Taiwan. However, *P. katsurae* has so far only been isolated from natural forests. Chestnut, coconut, and cacao, the only known hosts of *P. katsurae*, have been grown sporadically in Taiwan, but none of them were present in the forests surveyed. In fact, plants in the areas where *P. katsurae* and *P. heveae* were recovered in the protected forest at Lenhuachih all appeared healthy. It is conceivable that bath organisms may be able to colonize live or dead roots of certain plant species present in the forest without causing any visible symptoms. *Phytophthora cinnamomi* has been isolated from roots of various healthy-looking plants belonging to 28 species in 22 families on the island of Hawaii (Kliejunas and Ko, 1976).

Table 2. Oogonal protrusions of *Phytophthora katsurae* and *P. haveae*.

Species and isolate	No. of protrusions/oogonium	
	Range	Average
P. katsurae		
Pk-1	8-16	12
Pk-2	9-16	12
Pk-3	10-20	13
Pk-4	10-22	13
Pk-5	10-19	13
Pk-6	8-13	11
Pk-7	7-14	11
Pk-8	7-18	12
Pk-9	4-16	11
Pk-10	3-13	10
Pk-11	10-22	16
Pk-12	2-17	10
Pk-13	2-15	10
Pk-14	8-17	11
Pk-15	12-23	17
P. heveae		
Ph-1	0	0
Ph-2	0	0
Ph-3	0	0
Ph-4	0	0
Ph-5	0	0
Ph-6	0	0
Ph-7	0	0
Ph-8	0	0
Ph-9	0	0

Table 3. Oogonial protrusions of *Phytophthora katsurae* from Hawaii.

Isolate	No. of protrusions/oogonium		Oogonia without protrusion (%)
	Range	Average	
H1024	0-5	2.5	20
H1026	0-2	0.2	85
H1027	0-5	1.9	25
H1028	0-4	0.6	75
H1029	1-5	3.3	0
H1032	0-6	2.6	10

Species of *Phytophthora* require special chemicals for production of α hormones for formation of sexual organs such as oogonia (Ko, 1998; Chern et al., 1999; Jee et al., 2002; Wu et al., 2003). The verrucose oogonial wall is the morphological characteristic distinguishing *P. katsurae* from *P. heveae* (Ho et al., 1995; Stamps et al., 1990) However, this taxonomical characteristic appears to be unstable. Oogonia produced by isolates of *P. katsurae* from Taiwan contained protrusions ranging from 2 to 23 per oogonium. It is conceivable that *P. heveae* may have developed from *P. katsurae* through a gradual decrease in protrusion number. If the hypothesis is correct, it should be possible to find isolates of *P. katsurae* with very few protrusions on their oogonia. Six isolates of *P. katsurae* isolated from diseased coconut fruits in Hawaii were obtained from Dr. J. Y. Uchida (Uchida et al., 1992). All these isolates produced oogonia with very few protrusions (Table 3, Figure 2). Moreover, 75 to 85% of the oogonia produced by isolates H1028 and H1026 did not have any protrusions and were indistinguishable from those produced by *P. heveae* (Figure 2D). The ITS sequence similarity between *P. heveae* and *P. katsurae* (98.8%) was high in comparison with their relationships to other species tested (79.2 to 89.8%) (Table 4). These results clearly indicate that the two taxa share a recent common ancestor and that the presence of oogonial protuberances is a trait that distinguishes them.

Acknowledgements. We thank Drs. T. H. Chen and T. T. Chang of Taiwan Forestry Research Institute for assistance in collection of soil samples, Dr. Janice Uchida of the University of Hawaii for supplying the coconut isolates of *P. katsurae*, and Dr. Z. G. Zhang of Nanjing Agricultural University for ITS sequence similarity analysis.

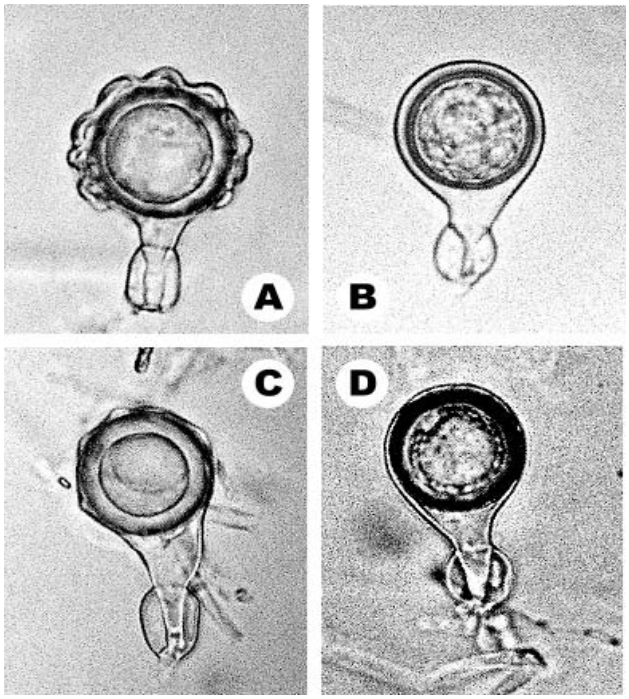

Figure 2. Comparison of oogonial morphology between *Phytophthora katsurae* (A) and *P. heveae* (B) from Taiwan, and *P. katsurae* from Hawaii (C, D). X 1000.

Table 4. Comparison of similarity levels of ITS sequences among *Phytophthora heveae*, *P. katsurae*, *P. humicola,* and *P. palmivora*[a].

Species paired		Similarity (%)
P. heveae	*P. katsurae*	98.8
P. heveae	*P. palmivora*	89.5
P. heveae	*P. humicola*	80.4
P. katsurae	*P. palmivora*	89.8
P. katsurae	*P. humicola*	83.1
P. humicola	*P. palmivora*	79.2

[a]ITS sequences were retrieved from GenBank.

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在台灣的自然保護林內所發現的 *Phytophthora katsurae* 同 *P. heveae* 之可能來源及關係

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從 1976 年到 2000 年由全台灣各縣採集的 531 個土壤中，只從三個縣分離到 16 株 *Phytophthora katsurae*，也只從四個縣分離到 9 株 *P. heveae*。在蓮花池的自然保護林內，我們同時分離到 *P. katsurae* 和 *P. heveae*。此保護林位於山頂上，附近視野內沒有比其更高的地方，由此可見此二種菌可能是台灣的原生種。*P. katsurae* 是以卵胞子囊壁突起，與很相近的 *P. heveae* 區別。台灣的 *P. katsurae*，每個卵胞子囊的突起數差異很大，由 2 個到 23 個，顯示此分類特性之不穩定。夏威夷分離到的 *P. katsurae* 菌株，所產生的卵胞子囊帶有很少的突起。而且其中兩株所產生的卵胞子囊，大部份都沒有突起，看起來與 *P. heveae* 的卵胞子囊沒有差別。這些的試驗結果顯示，*P. heveae* 可能由 *P. katsurae* 以消失卵胞子囊上的突起而發展出來。*P. heveae* 同 *P. katsurae* 的 ITS 的相似度，比他們同其他 *Phytophthora* 的相似度要高很多。這也支持他們之間，可能一個是由另一個於新近發展而來的觀點。

關鍵詞：*Phytophthora heveae*；*Phytophthora katsurae*；卵胞子囊突起；原生種。