
Molecular approach to clarify taxonomy of powdery mildew on Chilli plants caused by *Oidiopsis sicula* in Thailand

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Causal agent of powdery mildew on five chilli plants in Thailand viz.: *Capsicum frutescens*, *C. annuum* var. *grossum*, *C. frutescens* × *C. chinense* (Bhut Jolokia), *Capsicum* sp. (maxican chilli) and *Capsicum* sp. (darby chilli) has been identified as *Oidiopsis* sp. based on morphological data in Thailand. Molecular phylogenetic analysis indicated that the powdery mildew on *Capsicum* spp. is *Oidiopsis sicula* which supports the morphological data. This result confirmed that *Oidiopsis sicula* is linked to *Leveillula taurica* in teleomorph state. Maximum Parsimony tree showed that all sequence data are located in a clade consisted of *Leveillula taurica*, a fungal agent causing powdery mildew of *Capsicum* sp.

Key words: morphology, phylogeny, *Leveillula taurica*, *Capsicum* spp.

Introduction

The first systematic taxonomy of powdery mildews were studied based on morphological characteristics (Boesewinkel, 1980; Salmon, 1900; Ferraris, 1910). Some powdery mildews have similar morphological characteristics which cause confusing identification of the fungal group. In addition, sufficient information on morphological characteristics of sexual state (teleomorph) is essential to identify powdery mildews at species level. Unfortunately, most powdery mildews do not produce sexual state in tropical or sub-tropical areas. This is a problem for taxonomy of powdery mildew. Hirata and Takamatsu (1996) has been reported to use molecular analyze associate with anamorphic morphology in taxonomy of powdery mildew.

Nowadays, molecular technique is a useful tool for precise taxonomy for the Erysiphaceae. Khodaparast *et al.* (2001) determined the nucleotide

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sequences of the rDNA ITS regions for 13 *Leveillula* species on 50 host plant species and reported that the morphology of primary conidia mostly provides a good criterion to identify *Leveillula* species. This study also demonstrated that *L. taurica* s. lat. is a species complex composed of several biological species. Glawe *et al.* (2005) determined that powdery mildew on *Triglochin maritime* is caused by *L. taurica*. This result was confirmed by morphological and ITS sequence data. The ITS sequence of this fungus was identical with those reported for *L. taurica* hosted by *Capsicum annuum* in Australia and *Elaeagnus augustifolia* in Iran.

Chilli (Solanaceae) is an economic spice crop and cultivated commercially in all parts of Thailand (Poonpolgul & Kumphai, 2007). Powdery mildew on chilli is an important disease that causes yield losses in growing chilli area. And also, this disease is distributed in the other parts of the world (Palti, 1988). Sontirat *et al.* (1994) reported that *Oidiopsis* sp. is a causal agent of powdery mildew disease in Thailand. However, identification at species level was not shown because its perfect state has never been found on *Capsicum* species. The molecular analysis combined with morphological characteristics is a useful tool to approach for precise taxonomy of powdery mildews.

The present study was conducted to clarify the taxonomy of the fungal pathogen causing powdery mildew on chilli plants (*Capsicum* spp.) at species level on the basis of morphological data associate with molecular approach.

Materials and methods

Morphological observation

Specimens were collected in the northern Thailand since 2007. Fungal colonies on fresh specimens were stripped off by adhesive tape, mounted in distilled water and examined by standard light microscopy with 20X and 40X objective phase contrast lenses. Herbarium specimens were mounted in lactic acid, gently heated, but without any staining (Shin and La, 1993). Morphological characteristics were measured in 30 replicates for each structure: size and shape of conidia, conidiophore; position of the basal septum; shape and position of hyphal appressoria and presence or absence of fibrosin bodies (To-anun *et al.*, 2005). Observation of conidial germ tube was carried out using the method of Hirata (1942). Specimens were deposited in the mycological herbarium in Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Thailand and Mie University Mycological Herbarium (MUMH), Japan.

Phylogenetic analysis

Whole-cell DNA was extracted from mycelia or conidia using the chelex method (Walsh *et al.*, 1991; Hirata and Takamatsu, 1996). The nuclear rDNA ITS region including 5.8S rDNA was amplified by the polymerase chain reaction (PCR) using nested primer sets. The following thermal cycling conditions were performed in a PCR thermal cycler SP (Takara, Kyoto, Japan): an initial step for denaturing at 95°C for 2 min; thermocycling for 30 cycles that each cycle consisted of 30s at 95°C followed by 30s at 52°C for annealing, and 30s at 72°C for extension; and a final extension cycle at 72°C for 7 min. The oligonucleotide primers were used in this study as follows: ITS1, ITS4, ITS5, p3, PM6 and Ph7. A *Phyllactinia* and *Leveillula* specific primer Ph7 (TGTTGCTTTGGYAGGCCG) was designed in this study. Primers ITS5 (White *et al.*, 1990) and p3 (Kusaba and Tsuge, 1995) were used for the first amplification. Nested primer sets ITS5/PM6 and Ph7/ITS4 were used for the second amplification. The nucleotide fragments of PCR products were sent to SolGent Co. (Daejeon, South Korea) for sequencing by using ITS1 and ITS4 (White *et al.*, 1990) as sequence primers, respectively.

The nucleotide sequences of rDNA on ITS region were aligned with MUSCLE program (Edgar & Robert, 2004). Phylogenetic trees were constructed from data using maximum-parsimony (MP) analysis in MEGA5 (Tamura *et al.*, 2011) with a heuristic search using close-neighbor-interchange algorithm (CNI). All positions containing gaps and missing data were eliminated. The strength of the internal branches of the resulting trees was tested by bootstrap analysis (Felsenstein, 1985) using 1,000 replications. Lack of bootstrap value indicates less than 50% support at that node.

Result

Morphological observation

Powdery mildew was found on 5 chilli species, viz.: *Capsicum frutescens*, *C. annuum* var. *grossum*, *C. frutescens* × *C. chinense* (Bhut Jolokia), *Capsicum* sp. (maxican chilli) and *Capsicum* sp. (darby chilli). Diseased chilli plants appear symptom on leaves, but other parts of plant did not show symptoms. The lower side of leaves exhibited white-grayish colonies of fungi (hypophyllous) (Fig 1). The upper side of leaves showed a symptom as only yellow spot and then became to necrotic brown spot.

Mycelium hypophyllous; hyphae substraight to wavy, mostly branching near the septum; conidiophores erect, long and slender, arising from the upper part of mother cell, positions not central; foot-cells straight, with a basal septum

near branching point of mycelium up to away from it; appressoria slightly lobed to elongated; conidia formed singly, dimorphic conidia, apically pointed in primary conidia and ellipsoid to cylindrical in secondary conidia without conspicuous fibrosin bodies and conidial germination formed pseudoidium type (Fig 2). Chasmothecia can not be found. Table 1 showed size of morphological features of powdery mildew on each chilli species.

Phylogenetic analysis

The five rDNA sequences data on ITS region were aligned with 21 *Leveillula* sequences retrieved from GenBank. The alignment data matrix consisting of 26 taxa and 621 characters were used in the analysis. A total of 1,012 most parsimonious trees (CI = 0.708, RI = 0.832) were constructed by the MP analysis. One of MP tree is shown in Fig 3. The powdery mildew found on five chilli plants were located in the group of *L. taurica* causing powdery mildew on chilli under the accession numbers of AB000940 and MUMH3830.



Fig 1. *Capsicum annuum* var. *grossum* Bail. (sweet chilli) leaves showing a symptom of powdery mildew

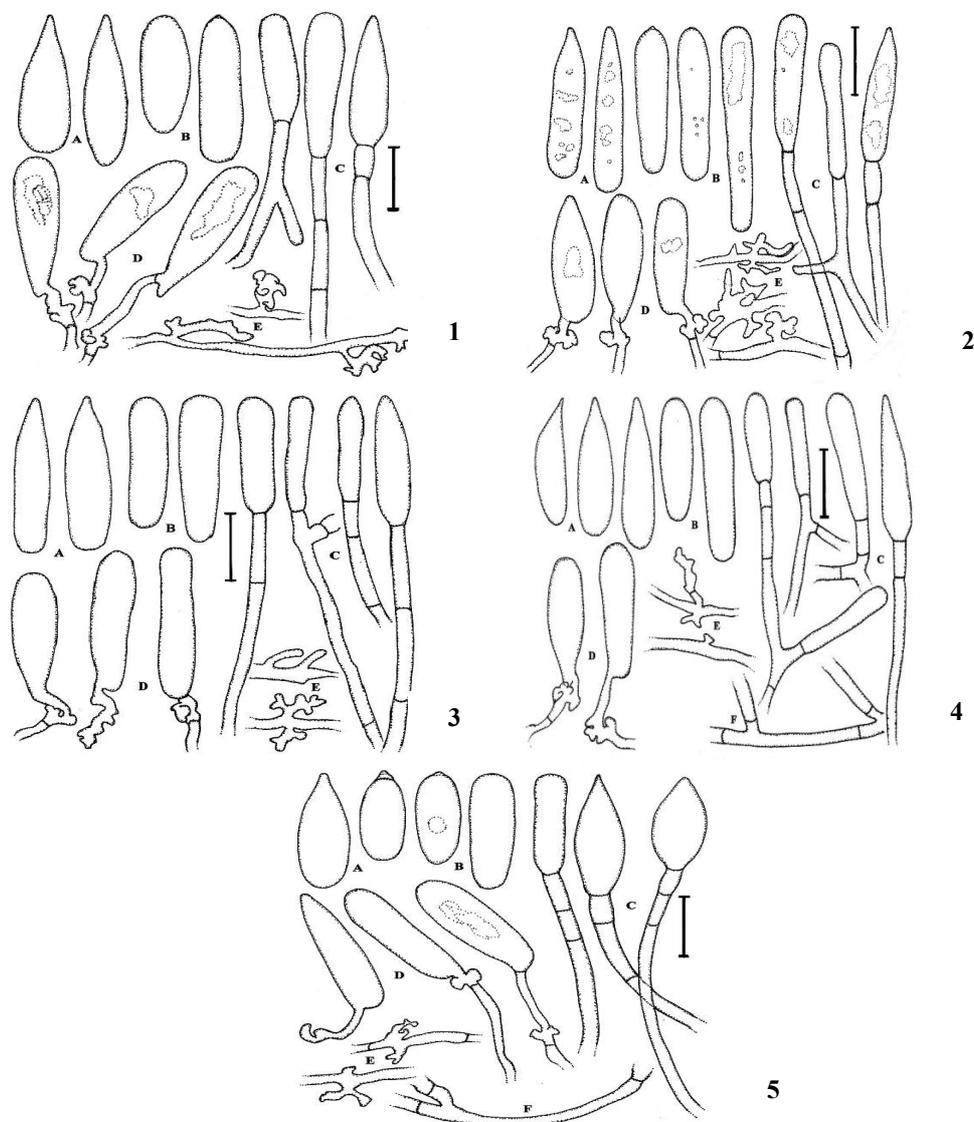


Fig 2. Morphological characteristics of *Oidiopsis* found on chilli plants illustrated using a line drawing under a light microscope (400X); (1) *Capsicum frutescens* (2) *C. annuum* var. *grossum* (3) *C. frutescens* × *C. chinense* (Bhut Jolokia) (4) *Capsicum* sp. (maxican chilli) (5) *Capsicum* sp. (darby chilli). Alphabet in figures described as follows; (A) primary conidia (B) secondary conidia (C) conidiophores (D) conidia with germ tubes of the pseudoidium pattern (E) mycelia with appressorium and (F) mother cells that originate of conidiophore (Bar = 30 µm.)

Table 1. Morphological characteristics of powdery mildew on chilli species.

Host	Size of morphological characteristics (µm)				
	conidiophore	mother cell	foot cell	primary conidia	secondary conidia
<i>Capsicum annuum</i> var. <i>grossumum</i> (MUMH5096)	(51-)100-276 (-338)×(5-)8-12(-16)	(28-)30-122 (-134)×5-6(-7)	(41-)49-140 (-202)×5-6(-7)	60-76(-83)× 13-17(-21)	(46-)52-69 (-78)×(12-)13-17
<i>Capsicum</i> spp. (maxican chilli) (MUMH5104)	(66-)133-266(- 281)×(10-)12-17	(24-)34-84(-107) ×5-6	(17-)48-137(-234) ×(4-)5-6(-7)	(61-)63-76(-78) ×(13-)15-19(-21)	(46-)58-73(-78) ×(13-)15-17(-19)
<i>Capsicum frutescens</i> (MUMH5083)	(105-)178-273(- 310)×(6-)8-15	(46-)49-83(- 151)×5-6	(56-)90-149(- 174)×5-7	(58-)63-80(- 88)×(15-)16-20	(49-)54-71(- 76)×(13-)15-19
<i>Capsicum</i> spp. (darby chilli) (MUMH5119)	(37-)110-251(-301)×(7-)8-12.2	(30-)35-89(- 100)×5-7	(44-)76-124(- 177)×5-7	(47-)58-73(- 74)×14-19	(41-)51-68(- 76)×(11-)13-18(- 19)
<i>C. frutescens</i> × <i>C. chinense</i> (Bhut Jologia) (MUMH5106)	(119-)144-195(- 278)×(7-)8-15(-17)	(22-)88-105(- 115)×(5-)6-7	(39-)71-107(- 149)×(5-)6-7	(54-)58-71(- 73)×(12-)15-18	(46-)5-72(-73)×(13-)15-17(-18)

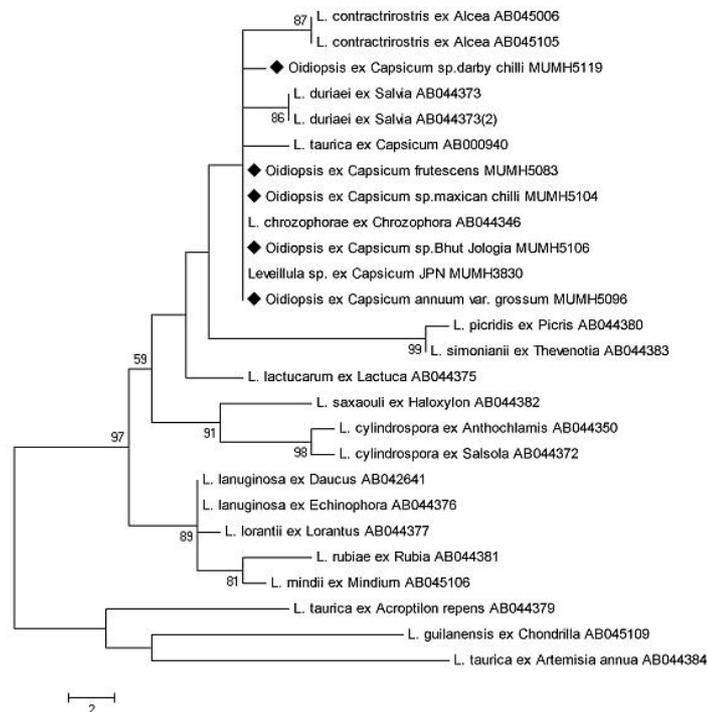


Fig 3. The Maximum parsimony phylogenetic tree based on fungal ITS gene sequences. Numbers above or below branches indicate bootstrap values (>50%) from 1,000 replicates. The tree length is 92, the consistency index (CI) is 0.708, the retention index (RI) is 0.832. Solid rhombus is represented as *Oidiopsis* that causing powdery mildew on chilli plants.

Discussion

The phylogenetic analysis represented by MP tree indicated that the five *Oidiopsis* specimens on chilli plants are located in a clade of *Leveillula* which confirms an anamorph-teleomorph connection of this fungus with *Leveillula*. *Leveillula* on *Acroptilon*, *Artemisia* and *Chondrilla* were used as the outer group based on Khodaparast *et al.* (2001).

The present phylogenetic result supported the morphological examination that showed no significance differences among five *Oidiopsis* found on *Capsicum* spp. (Braun, 1987 and Palti, 1988). Conidial germination type is *pseudoidium*-type (syn. *polygoni*-type) cited by Cook and Braun (2009). Thus, the morphological and phylogenetic analyses suggested strongly that the powdery mildew on *Capsicum* spp. is infected by *O. sicula* (teleomorph *L. taurica*) which agrees with the report of Goldberg (2004). Cunnington *et al.* (2003) revealed that *L. taurica* is a causal agent of powdery mildew disease on *C. annuum* in Australia. As a result, molecular analysis of rDNA ITS region is a strong tool to clarify taxonomy for species level in the genus. Species identification is an important information for accurate controlling this disease. In addition, this is the first report of taxonomy of powdery mildew on chilli plants by using morphological characteristics associated with molecular approach in Thailand.

This fungus attacks broadly range of plants (Glawe *et al.*, 2005; Khodaparast *et al.*, 2001). Future work such as pathogenicity test is necessary in order to determine pathogenicity of this fungus on differential varieties of chilli plants including other plants.

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