Cladosporioid on monocotyledon plant from Thailand

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Abstract During our studies on the diverse cladosporioid species occurring on monocotyledon plants, many species of Cladosporium previously unreported in Thailand were encountered. In this paper, we report on six species of Cladosporium from various monocotyledon plants. Based on analyses of the internal transcribed spacers (ITS1, 5.8S nrDNA, ITS2) and the translation elongation factor 1–α gene (EF–1α), the isolates are members of \textit{Cladosporium cladosporiodes} complex. Based on the cultural and micromorphological characteristics, they were identified as \textit{C. cladosporiodes}, \textit{C. cucumerinum}, \textit{C. perangusum}, \textit{C. pseudocladosporiodes}, \textit{C. tenuissimum} and \textit{C. xylophilum}. These six species represent new records in Thailand and world new host records.

Keys word: Cladosporioid, Cladosporium, monocotyledon

Introduction

Cladosporioid (Cladosporium–like) hyphomycetes are common, widespread fungi. The genus \textit{Cladosporium} is one of the largest, most heterogeneous genera of hyphomycetes, comprising more than 772 names (Dugan \textit{et al.}, 2004), and including endophytic, fungicolous, human pathogenic, phytopathogenic and saprobic species.

As plant pathogens, cladosporioid hyphomycetes cause severe impact on the growth of various economically important plants (Agrios, 2005). Since crop losses caused by fungal diseases pose a serious threat to global food/ security, it became apparent that the threat to agriculture from the deliberate release of pathogens, such as \textit{Cladosporium}, should not be underestimated. Nonetheless, the information of those phytopathogenic fungi in Thailand are quite limited

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and, therefore, still causing many difficulties for mycologist, plant pathologist, quarantine, and other scientific societies in Thailand to identify until species level. Almost no information, regarding this group of fungi and its distribution to the host plants specific in Thailand, are provided. Presently, only eight species of *Cladosporium* have been reported, namely, *Cladosporium cladosporioides* (Bensch *et al.*, 2010; Lumyong *et al.*, 2003), *C. colocasiae* (Manoch *et al.*, 1986), *C. musae* (Lumyong *et al.*, 2003), *C. oxysporum* (Lumyong *et al.*, 2003), *C. perangustum* (Bensch *et al.*, 2010), *C. sphaerospermum* (Sakayaroj *et al.*, 2010), *C. subuliforme* (Bensch *et al.*, 2010) and *C. tenuissimum* (Bensch *et al.*, 2010; Tokumasu *et al.*, 1990). Therefore, survey and research on diversity of other cladosporoid fungi in Thailand, and its distribution to the host plants are urgently needed.

The aims of this study are to provide a comprehensive database and a literature guide for the identification of cladosporoid fungi which are beneficial to support for the identification, detection, tracking, and risk assessment of this group of fungi.

**Materials and methods**

**Isolates**

Symptomatic monocotyledon leaves were collected at various locations in Chiang Mai, Thailand from 2011 to 2012. Symptomatic leaves were observed under stereomicroscope and incubated in moist chambers. Leaves were inspected daily for microfungi. Single–conidial isolates were obtained using techniques of Crous *et al.* (1991) and Crous (1998). Isolates were inoculated onto 2% potato–dextrose agar (PDA) at 25 °C to promote sporulation. All cultures obtained in this study were maintained in the culture collection of Plant Pathology Department, Chiang Mai University.

**Morphology**

Microscopic observations of the isolates were made from colonies cultivated for 7 d in 25 °C. Preparations from cultured fungal colonies were mounted on glass slides with clear lactic acid for microscopic examination. The study of conidial development and abranching patterns of conidial chains was done using the technique of Bench *et al.* (2010). Conidial terminology was followed from Schubert *et al.* (2007).
DNA isolation, amplification and sequence analysis

Fungal DNA extraction, PCR amplification and sequence analysis was patterned after Cheewangkoon et al. (2008). The Primers V9G (Hoog & Gerrits van den Ende, 1998) and LR5 (Vilgalys and Hester, 1990) were used to amplify part of the nuclear rDNA operon spanning the 3’ end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and the 5’ end of the 28S rRNA gene (LSU). The primers EF1–728F and EF1–986R (Carbone and Kohn, 1999) were used to amplify the translation elongation factor 1–α gene (EF–1α).

The generated sequences were compared with other fungal DNA sequences from NCBI’s GenBank sequence database using a blast search; sequences with high similarity were added to the alignments. The additional GenBank sequences were manually aligned using Sequence Alignment Editor v. 2.0a11 (Rambaut, 2002). The phylogenetic analyses of the aligned sequence data were performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford, 2003) with the heuristic search option using the ‘treebisection–reconstruction’ (TBR) algorithm. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of the internal branches of the resulting trees was tested with bootstrap analyses using 1000 replications (Felsenstein, 1985). Tree scores, including tree length, CI, RI, and RC, were also calculated.

Results and discussions

Phylogenetic analysis

The resulted alignment containing 62 taxa (including the outgroup taxon) and 412, 264 characters (including alignment gaps) were used in the ITS and TEF partitions, respectively. A total of 174 equally parsimonious trees with 710 steps (CI = 0.4873, RI =0.6951, RC = 0.3388) were generated by the parsimony ratchet analysis. One of the best trees is shown in Fig. 1.

The phylogenetic trees (Fig 1.) show that the Cladosporium species in this study segregate into 6 distinct clades, residing in the C. cladosporiodes complex clade. All represented species consisted of multiple strains are clustering in clades with bootstrap support values ranging from 60% (C. cladosporiodes) to 94% (C. cucumerinum).
**Taxonomy**

*Cladosporium cladosporioides* (Fresen.) G.A. de Vries. Fig. 2A.

Colonies on PDA were grey–olivaceous to dull green or olivaceous–grey. Conidiophores were straight to slightly flexuose, medium brown, (43–)51–69(–92) × (2.5–)3–3.5(–4) μm. Conidiogenous cells were integrated, usually terminal, pale brown, bearing branched conidial chains, (16–)24–26(29) × (2–)2.5–3(–3.5) μm. Ramoconidia were present, 1-2 cell, pale brown, with dimensions of (15–)18–27(–30)×(2.5–)3–3.5(–4) μm. Conidia were spherical to subpherical, smooth–walled, and the length and width of conidia were (3–)3.5–4(–4.5) × (2–)2.5–3(–3.5) μm.

**Notes:** Morphology of the fungus agreed well with the description of *C. cladosporioides* (Bensch et al., 2010). The species is morphologically closely related to *C. tenuissimum, C. cucumerinum* and *C. vignae* but differ in their pathogenicity to specific host plants (Bench et al., 2010). The pathogenicity test was not determined in this study. *C. cladosporioides* was reported from Thailand on *Areca* sp. (Bensch et al. 2010; 2012) and *Musa acuminata* (Lamyong et al. 2003). This study presented new its host plant including *Calamus siamensis, Hyophorbe verschaffeltii* and *Keriodoxa elegans*.

*Cladosporium cucumerinum* Ellis & Arthur Fig. 2B.

Conidiophores were straight to slightly flexuose, medium brown, (45–)61–75(–98) × (2–)2.5–3(–3.5) μm. Conidiogenous cells were integrated, usually terminal, pale brown, bearing branched conidial chains, (10–)12–22(36) × (2–)2.5–3(–3.5) μm. Ramoconidia were present, 1-2 cell, pale brown, with dimensions of (15.5–)18–x19(–24.5) × (2.5–)3–3.5(–4) μm. Conidia were spherical to subpherical, smooth–walled, and the length and width of conidia were (2–)2.5–3(–3.5) × (1.5–)2–2.5(–3) μm.

**Notes:** *Cladosporium cucumerinum* has similar conidiophore and conidium morphology which closely to *C. cladosporioides* and *C. vignae*. (Bensch et al., 2010). This study is the first report of *C. cucumerinum* from Thailand and also new host plants including *Saccharum officinarum, Zea maize, Equisetum debile, Phoenix loureiri* and *Livistona chinensis*.

*Cladosporium perangustum* Bensch, Crous & U. Braun Fig 2C.

Colonies on PDA were grey–olivaceous to dull green or olivaceous–grey. Conidiophores were straight to slightly flexuose, medium brown, (43–)51–69(–
92) \times (2.5–)3–3.5(–4) \, \mu m. Conidiogenous cells were integrated, usually terminal, pale brown, bearing branched conidial chains, (16–)24–26(29) \times (2–)2.5–3(–3.5) \, \mu m. Ramoconidia were present, 1-2 cell, pale brown, with dimensions of (15–)18–27(–30) \times (2.5–)3–3.5(–4) \, \mu m. Conidia were spherical to subpherical, smooth–walled, and the length and width of conidia were (3–)3.5–4(–4.5) \times (2–)2.5–3(–3.5) \, \mu m.

Notes: *Cladosporium perangustum* has been reported from Thailand on Acacia sp. (Bensch et al. 2010; 2012). This study provides the report of *C. cucumerinum* on new host plants including *Equisetum debile* and *Hymenocallis littoralis*.

**Cladosporium pseudocladosporioides** Bensch, Crous & U. Braun Fig 2D.

Colonies on PDA were grey–olivaceous to dull green or olivaceous–grey. Conidiophores were straight to slightly flexuose, medium brown, (20–)28–95(–112) \times (2.5–)3–3.5(–4) \, \mu m. Conidiogenous cells were integrated, usually terminal, pale brown, bearing branched conidial chains, (10–)12–36(44) \times (2–)2.5–3(–3.5) \, \mu m. Ramoconidia were present, 1-2 cell, pale brown, with dimensions of (8–)9–12(–13) \times (2–)2.5–3(–3.5) \, \mu m. Conidia were spherical to subpherical, smooth–walled, and the length and width of conidia were (6–)7–8(–9) \times (2–)2.5–3(–3.5) \, \mu m.

Notes: *Cladosporium pseudocladosporioides* has never been reported in Thailand. This study is the first in Thailand to report on this fungus which also has new host plants including *Cymbopogon citratus*, *Cyperus rotundus* and *Dactyloctenium aegyptium*.

**Cladosporium tenuissimum** Cooke Fig. 2E.

Colonies on PDA were grey–olivaceous to dull green or olivaceous–grey. Conidiophores were straight to slightly flexuose, medium brown, (43–)45–105(–127) \times (2–)2.5–3(–3.5) \, \mu m. Conidiogenous cells were integrated, usually terminal, pale brown, bearing branched conidial chains, (19–)23–33(41) \times (1–)1.5–2(–2.5) \, \mu m. Ramoconidia were present, 1-2 cell, pale brown, with dimensions of (10.5–)12–18(–19) \times (3–)3.5–4.5 (–5) \, \mu m. Conidia were spherical to subpherical, smooth–walled, and the length and width of conidia were (4–)4.5–5.5(–6) \times (2–)2.5–3(–3.5) \, \mu m.

Notes: *Cladosporiodes tenuissimum* was reported from Thailand on *Acaasia mangium*. (Bensch et al. 2010; 2012) and *Pinus khasya* (Tokumasu et al. 1990). This study presented new host plant including *Chamaedorea erumpeus* and *Cocos nucifera*. 

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**Cladosporium xylophilum** Bensch, Shabunin, Crous & U. Braun Fig. 2F.

Colonies on PDA were grey–olivaceous to dull green or olivaceous–grey. Conidiophores were straight to slightly flexuose, medium brown, (60–)72–86(–110) × (2–)2.5–3(3.5) μm. Conidiogenous cells were integrated, usually terminal, pale brown, bearing branched conidial chains, (20–)22–24(26) × (1–)1.5–2(–2.5) μm. Ramoconidia were present, 1-2 cell, pale brown, with dimensions of (18–)20–33(–36) × (2–)2.5–3(–3.5) μm. Conidia were spherical to subpherical, smooth–walled, and the length and width of conidia were (3–)3.5–4(–4.5) × (1–)1.5–2(–2.5) μm.

**Notes:** *Cladosporiodes xylophilum* was reported from only Europe and North America (Bensch *et al.* 2012). This study is the first report from Asia and with a new host plant, *Elaeis guineensis*.

**Table 1.** Isolates of *Cladosporium* used for DNA analysis and morphological studies.

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession number</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cladosporium cladosporiodes</em></td>
<td>AP013</td>
<td><em>Hyophorbe verschaffeltii</em></td>
</tr>
<tr>
<td></td>
<td>AP014</td>
<td><em>Keriodoxa elegans</em></td>
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<tr>
<td></td>
<td>AP023</td>
<td><em>Calamus siamensis</em></td>
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<tr>
<td><em>C. cucumerinum</em></td>
<td>AP021</td>
<td><em>Phoenix loureir</em></td>
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<tr>
<td></td>
<td>AP031</td>
<td><em>Zea maize</em></td>
</tr>
<tr>
<td></td>
<td>AP030</td>
<td><em>Saccharum officinarum</em></td>
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<tr>
<td></td>
<td>AP033</td>
<td><em>Equisetum debile</em></td>
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<tr>
<td></td>
<td>AP034</td>
<td><em>Livistona chinensis</em></td>
</tr>
<tr>
<td><em>C. perangusum</em></td>
<td>AP001</td>
<td><em>Hymenocallis littoralis</em></td>
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<tr>
<td></td>
<td>AP022</td>
<td><em>Cymbopogon citratus</em></td>
</tr>
<tr>
<td></td>
<td>AP027</td>
<td><em>Equisetum debile</em></td>
</tr>
<tr>
<td><em>C. psedocladosporiodes</em></td>
<td>AP024</td>
<td><em>Bambusa tulda</em></td>
</tr>
<tr>
<td></td>
<td>AP025</td>
<td><em>Cyperus rotundus</em></td>
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<td></td>
<td>AP026</td>
<td><em>Dactyloctenium aegyptium</em></td>
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<td>AP004</td>
<td><em>Chamaedorea erumeus</em></td>
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<tr>
<td><em>C. tenuissimum</em></td>
<td>AP005</td>
<td><em>Chamaedorea erumeus</em></td>
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<td><em>Cocos nucifera</em></td>
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<td><em>Cocos nucifera</em></td>
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<td>AP008</td>
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<td></td>
<td>AP009</td>
<td><em>Elaeis guineensis</em></td>
</tr>
<tr>
<td></td>
<td>AP017</td>
<td><em>Livistona chinensis</em></td>
</tr>
</tbody>
</table>

Fig. 1. One of 174 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined sequence alignment (ITS and EF). The scale bar shows five changes, and bootstrap support values from 1000 replicates are shown at the nodes. The tree was rooted to sequences of *Cercospora beticola* (CBS112.31).
Fig. 2. Cladosporium spp. Conidiophores, conidial chains and conidia. C. cladosporiodes (A), C. cucumerinum (B), C. perangusum (C), C. psedocladosporiodes (D), C. tenuissimum (E) and C. xylophilum (F). Scale = 20 (A, C–F), 10 (B) μm.

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References


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