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MYCOSCIENCE

ISSN 1340-3540 (print), 1618-2545 (online)

journal homepage: www.elsevier.com/locate/myc

Full paper

Molecular phylogeny reveals the presence of cryptic speciation within *Erysiphe japonica* (≡ *Typhulochaeta japonica*), a powdery mildew on *Quercus* spp.

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ARTICLE INFO

Article history:
Received 7 March 2012
Received in revised form
5 April 2012
Accepted 16 April 2012
Available online 26 September 2012

Keywords:
Erysiphaceae
Fagaceae
IGS
New variety
rDNA sequence

ABSTRACT

Molecular phylogenetic analyses based on 28S, ITS and IGS rDNA sequences indicate that *Erysiphe japonica* (≡ *Typhulochaeta japonica*) consists of two different genetic groups, one group on *Quercus aliena*, *Q. robur* and *Q. serrata*, and another group on *Q. crispula* var. *crispula* and *Q. crispula* var. *horikawae*. As morphological difference between the two groups are not yet marked distinctly, we suppose that the process of speciation has not yet been finished and propose a new variety, *E. japonica* var. *crispulae*, for the latter group based on the difference in host range and the distinct genetic segregation. *Quercus robur* (pedunculate oak) is a new host of *E. japonica*.

1. Introduction

Typhulochaeta S. Ito & Hara is a unique powdery mildew genus characterized by having clavate appendage-like cells arising from the upper half of chasmothecia (Ito 1915). These cells gelatinize in water and eject mucilaginous material. The first molecular phylogenetic analysis of *Typhulochaeta* was conducted by Mori et al. (2000), in which they demonstrated that *Typhulochaeta* nested in the *Erysiphe* clade (currently known as the tribe Erysipheae). Based on the molecular analysis, Braun and Cook (2012) re-allocated *Typhulochaeta* in the genus *Erysiphe* and proposed a new morphological, non-phylogenetic section, *Erysiphe* section *Typhulochaeta*.

Of the four species known in the section *Typhulochaeta* (Shin and Park 2011; Braun and Cook 2012), only *Erysiphe japonica* (S. Ito & Hara) C.T. Wei, the type species of *Typhulochaeta*, is distributed in Japan (Amano 1986). During preliminary phylogenetic analyses of *E. japonica*, we found that *E. japonica* is divided into two distinct groups dependent on host species (unpublished data). We thus conducted further phylogenetic analyses using additional specimens and DNA regions to confirm the result, and carried out detailed morphological observations of the specimens concerned. Consequently, we recognized the presence of cryptic speciation in *E. japonica*. *Quercus robur* (pedunculate oak) proved to be a new host of *E. japonica*.

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1340-3540/$ – see front matter
http://dx.doi.org/10.1016/j.myc.2012.07.003

2. Materials and methods

2.1. Morphological examination

Specimens were collected in several locations in Japan from 1995 to 2011. Details of host names, collection data, locations, and collectors were recorded. For morphological examinations, mycelia and chasmothecia were stripped off from the leaf surfaces with a clean needle, mounted on a microscope slide, and examined in 3% NaOH using a light microscope with phase contrast and 10×, 20×, and 40× objectives. Thirty chasmothecia, asci and ascospores were measured per sample. Specimens were deposited at the National Museum of Nature and Science (TNS), Japan, Mie University Mycological Herbarium (MUMH), Japan and Herbarium Martin-Luther-Universität, Halle (HAL), Germany.

2.2. Phylogenetic analysis

Whole-cell DNA was extracted from chasmothecia using the chelex method (Walsh et al. 1991) as described in Hirata and Takamatsu (1996). The 5-end of the 28S (including the domains D1 and D2), internal transcribed spacer including the 5.8S (ITS), and intergenic spacer (IGS) rDNA regions were amplified by polymerase chain reaction (PCR) using the respective primer pairs: PM3/TW14 (28S), PM7/ITS4 (ITS fragment 1), ITS5/PM6 (ITS fragment 2), and IGS-12A/NS1R (IGS; Carbone and Kohn 1999).

KOD FX Neo DNA polymerase (Toyobo, Japan) was used in the PCR reaction according to the manufacturer's protocol. The amplicons of 28S, ITS and IGS were sent to SolGent Co. Ltd. (Daejeon, South Korea) for sequencing using primers NL1 and NLP2 (28S), ITS1 and ITS4 (ITS), and IGS-12A and NS1R (IGS).

Representative sequences determined in this study were deposited in DNA databases (DDBJ, EMBL, GenBank) under the accession numbers of AB701300–AB701319. The Sequences were aligned with the sequences of *Erysiphe* species retrieved from DNA databases using MEGA 5 (Tamura et al. 2011). The alignment was deposited in TreeBASE (http://www.treebase.org/) under the accession number of S12462. Maximum parsimony (MP) analysis was done in PAUP* 4.0b10 (Swofford 2002) with the heuristic search option using the 'tree-bisection-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 tree was tested with bootstrap analysis using 1000 replications (Felsenstein 1985). Tree scores, including tree length, CI, RI, and RC, were also calculated.

3. Results

3.1. Taxonomy

Erysiphe japonica* var. *crispulae Meeboon & S. Takam., **var. nov.** Fig. 1.

MycoBank no.: MB 564666.

Morphologically similar to *E. japonica* var. *japonica* but confined to *Quercus crispula* var. *crispula* and *Q. crispula* var. *horikawae*.

Colonies on leaves hypophyllous, effuse or in large patches, persistent or sometimes evanescent. Hyphae 4–5 μm wide, hyaline. Chasmothecia scattered, blackish brown, (136–)148–191(–224) μm diam. (170 μm diam. on average, $n = 30$), containing 13–21 asci (Table 1). Peridial cells irregular in shape. Gelatinous cells numerous, relatively short, (24–)28–75(–87) × (8–)10–15(–19) μm (53 × 12 μm on average, $n = 30$), in the upper half of the chasmothecium, clavate, apically swollen or narrowed towards the tip, hooked, rarely straight, true appendages absent. Asci (67–)69–86(–91) × (29–)30–40(–44) μm (77 × 36 μm on average, $n = 30$), 7–8-spored, stalked. Ascospores (19–)21–25(–27) × (8–)10–13(–14) μm (23 × 11 μm on average, $n = 30$), ellipsoid-ovoid, hyaline; anamorph not developed.

Materials examined: on *Q. crispula* Blume var. *crispula* (Fagaceae), Japan, Shiga Prefecture, Mt. Odani, 7 November 1999, S. Takamatsu (Holotype: TNS-F-44918 Isotype: HAL2599F, MUMH 890). Additional specimens: Niigata Prefecture, Mt. Yahiko, 25 October 1997, S. Takamatsu (TNS-F-44920, HAL2501F, MUMH 427); Niigata Prefecture, Yuzawamachi, 23 September 1998, S. Takamatsu (TNS-F-44917, HAL2500F, MUMH 513); *Q. crispula* Blume var. *horikawae* H.Ohba, Japan, Toyama Prefecture, Asahi-cho, 26 June 1995, Y. Sato (MUMHs75).

Erysiphe japonica (S. Ito & Hara) C.T. Wei, Nanking J. 11(3): 105, 1942 (var. *japonica*) ≡ *Typhulochaeta japonica* S. Ito & Hara, Bot. Mag. Tokyo 29: 20, 1915.

Colonies on leaves amphigenous, mainly hypophyllous, effuse, forming large patches. Hyphae 3–5 μm wide, hyaline. Chasmothecia scattered, blackish brown, (143–)156–203(–217) μm diam. (181.5 μm diam. on average, $n = 30$), containing 11–22 asci (Table 1). Peridial cells irregular in shape. Gelatinous cells (30.5–)45–56(–62) × (10–)11–14.5(–16) μm (50 × 12.5 μm on average, $n = 30$), *ca* 100–160 per chasmothecium, in the upper half of the chasmothecium, clavate, apically swollen or narrowed towards the tip, straight, true appendages absent. Asci (69–)72–90(–103) × (28.5–)32–41.5(–45) μm (81 × 37 μm on average, $n = 30$), 8-spored, stalked. Ascospores (21.5–)22.5–28(–31) × (9.5–)10–14 μm (26 × 12.5 μm on average, $n = 30$), ellipsoid-ovoid, hyaline; anamorphs not developed.

Materials examined: on *Q. robur* L. (Fagaceae), Japan, Aichi Prefecture, Higashiyama Zoo and Botanical Gardens, 17 November 2011, J. Meeboon & S. Takamatsu (TNS-F-45899, HAL2502F, MUMH 5555); Osaka Prefecture, Botanical Garden of Osaka City University 20 November 2005, S. Takamatsu (TNS-F-44919, HAL2503F, MUMH 4146).

Notes: *Quercus robur* is reported here as a new host for *E. japonica*.

3.2. Phylogenetic analysis

In the 28S rDNA sequence analysis, 807 total characters were used, of which 765 characters were constant, 22 characters were variable and parsimony-uninformative and 20 characters were parsimony-informative. One equally parsimonious tree (Fig. 2) was generated from the MP analysis (TL = 44, CI = 0.955, RI = 0.967, RC = 0.923). In the ITS sequence analysis, 634 total characters were used including 523 constant characters, 78 variable and parsimony-uninformative characters, and 33 parsimony-informative characters. Two equally parsimonious trees (Fig. 3) were generated in this analysis