Identification of the pathogen *Podosphaera erigerontis-canadensis* causing powdery mildew disease on dandelion (*Taraxacum officinale*) in US Arkansas state

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Abstract

Powdery mildew was observed on common dandelion (*Taraxacum officinale*), collected in Fayetteville, AR, USA, and was identified using morphology and DNA sequences of ITS and 28S ribosomal RNA (rRNA) gene regions. Four primer pairs were used in this study: (1) the powdery mildew specific primer pair PMITS1/2 produced a 696 bp DNA fragment including full sequence of 563 bp DNA fragment amplified by ITS1/4 (KU563734); (2) CTW13/TW14 created a 279 bp DNA fragment including partial sequence of 28S rRNA gene (KU563735); (3) the 28S rRNA region primer pair NL1f/TW14 amplified a 899 bp DNA fragment (KX610821); and (4) the primer pair ITS1/4 amplified a 563 bp DNA fragment including partial sequence of 18S ribosomal RNA gene and complete sequence of ITS1, the 5.8S rRNA gene, ITS2, and partial sequence of the 28S rRNA gene. The similarity analysis by Blast and phylogenetic analysis indicated that the powdery mildew pathogen in dandelion collected from Arkansas was *Podosphaera erigerontis-canadensis*.

Keywords Dandelion · Internal transcribed spacer (ITS) · *Podosphaera erigerontis-canadensis* · Powdery mildew · *Taraxacum officinale*

Powdery mildew is one of the most common and widespread plant diseases (Ale-Agha et al. 2013). Most powdery mildew diseases are caused by the Erysiphaceae family which consists of 16 genera and approximately 900 species (Braun and Cook 2012). *Erysiphe* is the largest genus in Erysiphaceae (Takamatsu et al. 2015), followed by *Golovinomyces* and *Podosphaera*. Although powdery mildew disease is caused by different species and occurs on a wide range of plants, the symptoms of powdery mildew are similar: white to greyish spots, and powder-like cover on leaves, stems, and sometimes fruit. Powdery mildew usually affects young tissue, including young leaves. The white spots gradually spread to cover most

of the leaves, and finally leaves turn yellow and dry out. Morphological criteria and host ranges are the classical methods to identify fungal pathogens. In recent decades, internal transcribed spacer (ITS) as a universal DNA barcode marker have been used for fungal diagnostics and phylogeny (Schoch et al. 2012). The study of biology and taxonomy of fungi is efficient ways to understand the species of powdery mildews in a geographic region (Khodaparast and Abbasi 2009). Molecular re-examination of *Podosphaera fusca* resulted in delineation of *Podosphaera erigerontis-canadensis* on *T. officinale* (Takamatsu et al. 2010) and morphologically described *P. fusca* on *Conyza canadensis* in France (Braun and Cook 2012), *Taraxacum* sp. in Iran (Sharifi et al. 2014) and *Crepis* sp. and *Erigeron* sp. in Sweden (Eriksson 2014). Prior to taxonomic revisions of powdery mildew species, *P. erigerontis-canadensis* was reported under the name *Sphaerotheca erigerontis-canadensis*, *S. fuliginea*, *S. fusca*, *S. macularis* and *S. humuli* on a range of hosts including *T. officinale* in the USA (Farr et al. 1989; Braun et al. 2013) and worldwide. However, there are no records of this fungus colonising *T. officinale* in Arkansas, the USA. Since 2014, the Vegetable Breeding program at the University of Arkansas has started a new research project on common dandelion, and the

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Fig. 1 The symptom of powdery mildew disease on common dandelion leaf

purpose is to develop common dandelion as a vegetable. Therefore, the present research aimed to investigate the powdery mildew species on dandelion.

Powdery mildew samples were collected from dandelion leaves with obvious powdery mildew symptoms (Fig. 1), grown in the fields in Fayetteville, Arkansas, United States in fall 2014, 2015 and 2016. White or greyish white powdery spots developed on leaves and stems, and mycelia on leaves was effused or were in irregular patches. Mycelia covered almost the entire leaf surface. Old mycelia usually turned brownish. Conidiophores were straight, 220–320 μm long, 10–12 μm wide with 4 to 6 immature conidia in chains. Ovoid shape of conidia was 45–60 μm long, 10–15 μm wide

with distinct fibrosin bodies. Unbranched germ tube was seen from the lateral position of conidia. Chasmothecia were subglobose, 60–80 μm diameter, and scattered in patches. Ascus was oblong shape, 60–80 μm long, 40–55 μm wide, enclosed in chasmothecia. They were characterised by smaller ascomata and ascus with smaller terminal oculus compared to *Podosphaera fusca* (Braun and Cook 2012) (Fig. 2). 6–8 ellipsoidal ascospores were inside of each ascus, which were 14–22 μm long, and 8–11 μm wide. The symptoms and characters of the teleomorph of this powdery mildew pathogen on dandelion were similar to that of *Podosphaera erigerontis-canadensis* described by Braun and Liu (2010). Voucher specimen is deposited in the Cryptogamic Herbarium of the New

Fig 2 *Podosphaera erigerontis-canadensis* from dandelion leaves: (a) conidia and conidiophores on the leaf, (b) conidia, (c) chasmothecia on the leaf, and (d) chasmothecium with ascus and ascospores

York Botanical Garden, Bronx, New York, USA (accession number NY 03103415).

DNA was extracted from mycelia and conidia using the CTAB protocol (Shi and Mmbaga 2006; Takamatsu et al. 2010). Polymerase chain reactions (PCRs) were performed using four pairs of primers to amplify ITS and 28S rDNA regions: (1) powdery mildew specific ITS primers PMITS1 (5'-tcggactggcccaggaga-3')/PMITS2 (5'-tcactcgccgttactgaggt-3') (Cunnington et al. 2003), (2) CTW13 (5'-cgtcttgaaacacggacc-3')/TW14 (5'-gctatcctgagggaaacttc-3') (source: <http://nature.berkeley.edu/brunslab/tour/primers.html>), (3) NL1f (5'-tgggtggtaaattccatcta-3')/TW14 (White et al. 1990; source: <http://nature.berkeley.edu/brunslab/tour/primers.html>), and (4) universal ITS primers ITS1 (5'-tccgtaggtgaacctgcgg-3')/ITS4 (5'-tcctccgcttattgatgc-3')

(White et al. 1990). PCR products were purified by using OMEGA E.Z.N.A.® Cycle Pure Kit. The purified DNA products were sequenced from 5' and 3' directions by Eurofins Genomics Company, and consensus sequences were assembled and used for subsequent phylogenetic analyses.

Three DNA sequences were deposited in GenBank with the accession numbers, KU563734, KU563735, and KX610821. KU563734 was amplified by the primer pair PMITS1/2 with a 696 bp size of DNA fragment; KU563735 by CTW13/TW14 with a 279 bp DNA fragment; and KX610821 by NL1f/TW14 with a 899 bp DNA fragment. Besides, a 563 bp DNA fragment was also amplified by the primer pair ITS1/4, but it was not published in GenBank because it was part of KU563734. This DNA fragment of KU563734 included the ITS region, complete sequence of

Fig. 3 Phylogenetic tree based on the ITS sequences for *Podosphaera erigerontis-canadensis* identified from dandelion in Fayetteville, Arkansas, United States, and 39 ITS sequences of fungi causing powdery mildew in different plants published in GenBank

Fig. 4 Phylogenetic tree based on the 28S rDNA sequences for *Podosphaera erigerontis-canadensis* identified from dandelion in Fayetteville, Arkansas, United States, and 19 28S rDNA sequences of fungi causing powdery mildew in different plants published in GenBank

ITS1, complete sequence of 5.8S rRNA gene, complete sequence of ITS2, and partial sequence of 28S rRNA gene. KU563735 and KX610821 covered partial 28S rRNA gene. The results of BLAST search analysis indicated that the three sequences of KU563734, KU563735, and KX610821 had 100% identity to *P. erigerontis-canadensis* (GenBank: AB525916) (Figs. 3 and 4), which was identified in dandelion (*T. officinale*) from Argentina (Takamatsu et al. 2010). Based on morphology and DNA BLAST search, the pathogen causing powdery mildew on dandelion collected from Arkansas is *P. erigerontis-canadensis*, which was the same pathogen for powdery mildew affecting dandelions in Argentina.

For similarity analysis to other pathogens of powdery mildew, a seven nucleotide difference was found after comparison with the sequence of *Podosphaera xanthii* (GenBank: KM260741), and eight nucleotides were identified to be different from the sequence of *Podosphaera balsaminae* (GenBank: KR048106). Other 28S rDNA sequences of powdery mildew fungi in GenBank including *Podosphaera xanthii* (GenBank: AB462760), *Podosphaera balsaminae* (Genbank: AB462789), *Podosphaera diclipterae* (GenBank: AB462795), *Podosphaera euphorbiae-hirtae* (GenBank: AB462770), *Podosphaera cayratiae* (GenBank: AB462765), and *Podosphaera astericola* (GenBank: AB462779), found that the DNA sequence amplified by the primer NL1f/TW14 from the powdery mildew fungus identified in Arkansas, USA had one nucleotide difference, further indicating that the *P. erigerontis-canadensis* was a distinct species causing powdery mildew on dandelion in Arkansas.

In this study, a total of 39 ITS and 19 28S sequences of powdery mildew pathogens were used for phylogenetic analysis by using MEGA 6 (Tamura et al. 2013) aligned by MUSCLE method. The results, shown in Figs. 3 and 4, indicated that there were no phylogenetic difference between the fungal pathogen found in Fayetteville, Arkansas, United States and in Argentina by Takamatsu (GenBank: AB525916).

This species has not been documented in Arkansas, the USA. Other powdery mildew species reported on dandelion in the USA were *Sphaerotheca humuli*/S. *humuli* var. *fuliginea* (Plant Pathology Database, University of Massachusetts Amherst- https://urldefense.proofpoint.com/v2/url?u=https-3A__www.bio.umass.edu_biology_research_herbarium_plantpathology_archive.php-3Fgenus-3DSphaerotheca&d=DwIGaQ&c=7ypwAowFJ8v-mw8AB-SdSueVQgSDL4HiiSaLK01W8HA&r=f2dIVbIi-OnCTqImm6VdMg&m=_tIJuLcWz6_4ztydi15mP11WbzBYxlrRsB2gT2hL_E&s=rUj7bhFfnyGgp4kcAsYqald1WXCywgddunTSLZ5P6tM&e=), *Golovinomyces cichoracearum* and *Phyllactinia guttata* (Farr et al. 1989). Identification of these species was based on morphological markers that are distinct from the morphological markers associated with *P. erigerontis-canadensis*. *Oidium* subgenus *Fibroidium* was also reported on dandelion in Japan (Satou et al. 2012) and its identification was based on the sequencing of the ITS region. Our DNA sequencing data and phylogenetic analysis indicate that this is the first report of *P. erigerontis-canadensis* on dandelion in Arkansas, the USA.

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References

- Ale-Agha N, Boyle H, Braun U, Butin H, Jage H, Kummer V, Shin HD (2013) Taxonomy, host range and distribution of some powdery mildew fungi (*Erysiphales*). *Schlechtendalia* 17:39–54
- Braun U, Cook RT (2012) Taxonomic manual of the *Erysiphales* (powdery mildews). CBS-KNAW Fungal Biodiversity Centre, Utrecht, p 707
- Braun U, Liu T (2010) The *Erysiphaceae* of Inner Mongolia. Inner Mongolia Science and Technology Press, Chifeng, p 198
- Braun U, Shishkoff N, Takamatsu S (2013) Phylogeny of *Podosphaera* sect. *Sphaerotheca* subsect. *Magnicellulatae* (*Sphaerotheca fuliginea* auct. s.lat.) inferred from rDNA ITS sequences – a taxonomic interpretation. *Schlechtendalia* 7:45–52
- Cunnington JH, Takamatsu S, Lawrie AC, Pascoe IG (2003) Molecular identification of anamorphic powdery mildews (*Erysiphales*). *Australas Plant Pathol* 32:421–428
- Eriksson E (2014) Checklist of the non-lichenized ascomycetes of Sweden. *Acta Univ Upsal Symb Bot Upsal* 36:499
- Farr DF, Bills GF, Chamuris GP, Rossman AY (1989) Fungi on plants and plant products in the United States. APS press p 1225
- Khodaparast SA, Abbasi M (2009) Species, host range and geographical distribution of powdery mildew fungi (Ascomycota: *Erysiphales*) in Iran. *Mycotaxon* 108:213–216
- Satou M, Chikuo Y, Matsushita Y (2012) Powdery mildew of dandelion (*Taraxacum officinale*) caused by *Oidium* subgenus *Fibroidium*. *Annual Report of the Kanto-Tosan Plant Protection Society* 59: 77–78
- Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Miller AN (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *PNAS* 109(16):6241–6246
- Sharifi K, Davari M, Khodaparast SA, Bagheri-Kheirabadi M (2014) A study on the identification of powdery mildew fungi (*Erysiphaceae*) in Ardabil landscape. *J Crop Prot* 3:663–671
- Shi A, Mmbaga MT (2006) Perpetuation of powdery mildew infection and identification of *Erysiphe australiana* as the crape myrtle pathogen in mid-Tennessee. *Plant Dis* 90:1098–1101
- Takamatsu S, Niinomi S, Harada M, Havrylenko M (2010) Molecular phylogenetic analyses reveal a close evolutionary relationship between *Podosphaera* (*Erysiphales*: *Erysiphaceae*) and its rosaceous hosts. *Persoonia* 24:38–48
- Takamatsu S, Ito H, Shiroya Y, Kiss L, Heluta V (2015) First comprehensive phylogenetic analysis of the genus *Erysiphe* (*Erysiphales*, *Erysiphaceae*) I. The *Microsphaera* lineage. *Mycologia* 107:475–489
- Tamura K, Stecher G, Peterson D, Ailipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729
- White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, pp 315–322