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Mini Review

Molecular characterization and phylogenetic analysis of *Colletotrichum* species associated with anthracnose disease

Abstract

Colletotrichum species are related with anthracnose of a wide-ranging of host plants containing cultivated and wild tropical fruits. The genetic and ecological variety of species connected with wild fruits exist poorly explored, as associated to those affiliated with pre and post harvest diseases of cultivated fruits. It is needed to re-assess the evolutionary relationships of *Colletotrichum* species arising in cultivated and wild fruits with prominence on their ecology and cryptic divergence containing sampling at regional and global scales. This study we will examine to analysis of sequences of nuclear ribosomal internal transcribed spacer (ITS).To observe phylogenetic associations and closeness within *Colletotrichum* species. Multi-locus phylogenetic analysis of strains of the *Colletotrichum* complex associated with anthracnose.

Keywords: *colletotrichum*, harvest diseases, ecological, tropical, cultivated fruits

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Introduction

Colletotrichum is an important plant pathogenic genus affecting anthracnose of a widespread assortment of vegetables, fruits cereals, grasses and ornamental plants in temperate tropical and regions.[1–3] Fruit production is mostly affected in both high-value crops and wild fruits in natural habitats. However, *Colletotrichum* species associated with wild fruits are poorly known.[4,5] *Colletotrichum* specie was voted as the eighth most important plant pathogens in the world in a recent survey among fungal pathologists, for its perceived scientific and economic importance.[6] The fruits infected by *Colletotrichum* have small, water-soaked, sunken, circular spots that may increase in size with age and the center of an older spot becomes blackish and develops gelatinous pink or orange spore masses.[7–9] Recent studies have focused on phylogenetic re-assessments of species complexes,[10] and have determined that what were previously thought to be a single species, comprise multiple distinct lineages.

For example the boninense clade (*Colletotrichum boninense* species complex) now comprises about 18 species,[11] while the acutatum clade (*C. acutatum* species complex) now comprises 31 species[11] and the *gloeosporioides* clade (*C. gloeosporioides* species complex) comprises more than 22 species.[12] The species numbers in the major clades are likely to rise, unraveling the cryptic taxa based on multi-gene phylogenetic analyses and incorporating a large number of isolates in comprehensive collections.[10] In addition to the major species complexes in *Colletotrichum*, several intermediate clades have studied. Epitypification of *Colletotrichum gloeo sporioides,*[13] and subsequent use of multi-gene phylogeny have resulted in this taxon being revealed as a species complex. *Colletotrichum* gloeo sporioides was originally described from Citrus in Italy, thus the chosen epitope culture derived from a necrotic spot on leaves of Citrus sinensis from the same country.[13] *Colletotrichum gloeosporioides* was previously thought to be a cosmopolitan species infecting a broad range of plant hosts including tropical fruits,[14–16] tested this hypothesis by molecular and morphological characterization of *Colletotrichum* strains from anthracnose symptoms on tropical fruits in Laos and Thailand. *Colletotrichum gloeosporioides* sensu stricto was not found from any of the fruit examined in their study, however many strains from various common fruits were not assigned to any known taxa based on the five genes employe.[17] studied the large subunit of the nuclear ribosomal RNA gene (LSU) and the interior tran- scribed spacer 2 of the nuclear ribosomal RNA operon (ITS- 2) of *Colletotrichum* separates from many hosts. For a reason that of the uniformity of the DNA sequence data they established *C. orbiculare, C. lindemuthianum, C. malvarum, and C. trifolii* to be unique type and recommended the name *C. orbiculare* in which host specific forms exist. However, formed on sequence data of the glutamine synthase gene (GS) and a 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH), DNA restriction fragment distance polymorphisms (mtDNA RFLP) Mitochondrial and vegetative compatibility as well as pathogenicity tests of a huge number of strains[18] identified *C. orbiculare* as a species complex with, *C. malvarum, C. orbiculare, C. lindemuthianum* and *C. trifoliias* distinct species. *Colletotrichum* orbiculare cause anthracnose of *Cucurbitaceae* and is phylogenetically thoroughly related to pathogens of many additional herbaceous hosts belonging to the *Asteraceae, Fabaceae* and *Malvaceae.*[19] Maximum of them are recognized for their hemibiotrophic infection plan and as destructive pathogens either of area crops or weeds. To study the phylogenetic associations of these fungi, a multilocus analysis (ITS, CHS-1 GAPDH, ACT, HIS3, TUB2, GS) of 42 strains of *C. orbiculare* and associated species conducted. The analysis resulted in nine clades that confirmed the four species formerly known as belonging to this species complex, *C. lindemuthianum, C. malvarum, C. orbiculare* and *C. trifolii*, and recognized four novel species from weeds, i.e. *C. bidentis, C. sidae, C. spinosum and C. tebeestii. Colletotrichum gloeosporioides* was earlier regarded as the first *Colletotrichum* species to infect species of the Proteaceae.[20] Based on morphology, sequence data of the interior transcribed insertion region (ITS) and partial sequences of the Beta-tubulin gene (TUB2),[21] distinguished four species of *Colletotrichum* (*C. acutatum, C. boninense, C. crassipes, C. gloeosporioides*, *C. acutatum*) related with diseased *Proteaceae*. An additional strain recognized as *C. gloeosporioides* based on ITS and 28S rDNA gene (LSU) sequence data was involved in the study.[22]

Isolation of fungi and its morphological detection and observation

Strains of *Colletotrichum* is collected from forest trees and different cultivated fruiting plant species i.e. pepper, apple, banana, mango etc Cultures will firstly maintain on potato dextrose agar (PDA) before the observation of colony characters and growth rates. Colony characters i.e. Colony diameter, colony size, shape, and color is record (Figure 1).

Figure 1 Symptoms of different color pepper anthracnose in the field (Fangling Liu et al 2016).

DNA extraction and sequencing

Isolates is grown on PDA and incubated for 5days at 25°C in the dark. DNA will be extracted using the protocol as outlined,[23] using the actively growing mycelia from the edge of cultures. The ITS region will be sequenced using the forward/reverse primer pair ITS5/ITS4 and then on this basis amplification and sequencing of multiple gene regions will be done. All PCR products will then be visualized on 1% agarose gel. PCR products will then be purified and sequenced (Figure 2).

Figure 2 Colonies of *Colletotrichum* species on PDA.

Phylogenetic analyses

It is performed either on the multi-locus alignment (ACT, CAL, GAPDH, CHS-1, GS, ITS, TUB2) using PAUP v.4.0b10,[24] or using a Markov Chain Monte Carlo (MCMC) algorithm to make trees or both of the processes is applied. Sequences derived in this study were deposited in GenBank, the concatenated alignment in TreeBASE (www.treebase.org), and taxonomic novelties in MycoBank.

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Conflict of interest

The author declares no conflict of interest.

References

1. Sreenivasaprasad S, Brownm AE, Mills P. DNA sequence variation and interrelationships among *Colletotrichum species* causing strawberry anthracnose. *Physiological and Molecular Plant Pathology*. 1992;41(4):265–281.
2. Freeman S, Horowitz S, Sharon A. Pathogenic and nonpathogenic lifestyles in *Colletotrichum* acutatum from strawberry and other plants. *Phytopathology*. 2001;91(10):986–992.
3. Rojas EI, Rehner SA, Samuels GJ, et al. *Colletotrichum gloeosporioides* associated with Theobroma cacao and other plants in Panama: multilocus phylogenies distinguish host–associated pathogens from asymptomatic endophytes. *Mycologia*. 2010;102(6):1318–1338.
4. Rampersad SN. Molecular and phenotypic characterization of *Colletotrichum species* associated with anthracnose disease of papaya in Trinidad. *Plant disease*. 2011;95(10):1244–1254.
5. Cannon PF, Damm U, Johnston PR, et al. *Colletotrichum*–current status and future directions. *Studies in mycology*. 2012;73(1):181–213.
6. Yang HC, Haudenshield JS, Hartman GL. First report of *Colletotrichum* chlorophyti causing soybean anthracnose. *Plant Disease*. 2012;96(11):1699–1699.
7. Cai L, Hyde KD, Taylo PWJ, et al. A polyphasic approach for studying *Colletotrichum*. *Fungal Diversity*. 2009;39(1):183–204.
8. Gautam AK. The genera *Colletotrichum*: an incitant of numerous new plant diseases in India. *Phytopathology*. 2005;58(1):125.
9. Nelson SC. *Mango anthracnose (Colletotrichum gloeosporiodes)*. 2008.
10. Damm U, Cannon PF, Woudenberg JH, et al. The *Colletotrichum* acutatum *species* complex. *Stud Mycol*. 2012;73(1):37–113.
11. Damm U, Cannon PF, Woudenberg JH, et al. The *Colletotrichum boninense species* complex. *Stud Mycol*. 2012;73(1):1–36.
12. Weir BS, Johnston PR, Damm U. The *Colletotrichum gloeosporioides species* complex. *Stud Mycol*. 2012;73(1):115–180.
13. Cannon PF, Buddie AG, Bridge PD. The typification of *Colletotrichum gloeosporioides*. *Mycotaxon*. 2008;104:189–204.
14. Alahakoon P, Brown A, Sreenivasaprasad S. Cross–infection potential of genetic groups of *Colletotrichum gloeosporioides* on tropical fruits. *Physiological and Molecular Plant Pathology*. 1994;44(2):93–103.
15. Abang M, Winter S, Green KR, et al. Molecular identification of *Colletotrichum gloeosporioides* causing yam anthracnose in Nigeria. *Plant Pathology*. 2002;51(1):63–71.
16. Phoulivong, S, Lei C, Hang C, et al. *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. *Fungal Diversity*. 2010;44(1):33–43.

17. Sherriff C, Whelan MJ, Arnold GM, et al. Ribosomal DNA sequence analysis reveals new *species* groupings in the genus *Colletotrichum*. *Experimental mycology*. 1994;18(2):121–138.
18. Liu G, Kennedy R, Greenshields DL, et al. Detached and attached Arabidopsis leaf assays reveal distinctive defense responses against hemibiotrophic *Colletotrichum spp. Mol Plant Microbe Interact*. 2007;20(10):1308–1319.
19. Wang B, Yan Y, Tian Y, et al. Heterologous expression and characterisation of a laccase from *Colletotrichum lagenarium* and decolourisation of different synthetic dyes. *World J Microbiol Biotechnol*. 2016;32(3):40.
20. Baxter AP, Westhuizen GV, Eicker A. Morphology and taxonomy of South African isolates of *Colletotrichum*. *South African Journal of Botany*. 1983;2(4):259–289.
21. Lubbe CM1, Denman S, Cannon PF, et al. Characterization of *Colletotrichum species* associated with diseases of Proteaceae. *Mycologia*. 2004;96(6):1268–1279.
22. Liu F, Damm U, Cai L, et al. *Species* of the *Colletotrichum gloeosporioides* complex associated with anthracnose diseases of Proteaceae. *Fungal Diversity*. 2013;61(1):89–105.
23. Udayanga D, Xingzhong L, Pedro WC, et al. A multi–locus phylogenetic evaluation of Diaporthe (Phomopsis). *Fungal Diversity*. 2012;56(1):157–171.
24. Liu F, Cai L, Crous PW, et al. The *Colletotrichum* gigasporum *species* complex. *Persoonia*. 2014;33:83–97.

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