British Mycological Society promoting fungal science

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

Molecular identification of two strains of *Cercospora rodmanii* isolated from water hyacinth present in Yuriria lagoon, Guanajuato, Mexico and identification of new hosts for several other strains

José Guadalupe MONTENEGRO-CALDERÓN[a], *José Ascención MARTÍNEZ-ÁLVAREZ*[a], *Ma. Teresa VIEYRA-HERNÁNDEZ*[a], *Luz Imelda RANGEL-MACÍAS*[a], *Tannia RAZZO-SORIA*[a], *Roberto CHÁVEZ-HERRERA*[b], *Patricia PONCE-NOYOLA*[a], *Carlos Alberto LEAL-MORALES*[a,*]

[a]*Departamento de Biología, División de Ciencias Naturales y Exactas, Campus Guanajuato, Universidad de Guanajuato, Noria Alta s/n Guanajuato, Gto, C.P. 36050, Mexico*

[b]*Secretaría de Desarrollo Agropecuario del Estado de Guanajuato, Av. Irrigación s/n, Celaya, Gto, C.P. 38010, Mexico*

ARTICLE INFO

Article history:
Received 22 March 2010
Received in revised form
10 August 2011
Accepted 15 August 2011
Available online 28 August 2011
Corresponding Editor:
David E.L. Cooke

Keywords:
Actin
β-Tubulin
Calmodulin
Histone H3
nrRNA
Translation elongation factor1-α

ABSTRACT

Water hyacinth is a beautiful monocotyledon plant that has been dispersed all over the world by humans. The plant has been present in Mexico since 1907, and many water bodies have become infested with it since then. In 2001, we initiated a survey in Yuriria lagoon in southern Guanajuato state to isolate fungi able to biocontrol the plant. We isolated 25 morphologically distinct fungal cultures, of which two were identified as members of the genus *Cercospora*. *Cercospora* species are among the most prevalent and destructive of plant pathogens and can be found on leaves, pedicels, stems, fruits, and bracts. Only two species of *Cercospora*, *Cercospora piaropi*, and *Cercospora rodmanii*, have been described on water hyacinth; however, the classification of these species has been controversial. Several molecular approaches have been used for *Cercospora* identification, and some candidate genes have been identified for use in *Cercospora* species determination. Although the nrRNA genes alone do not show sufficient resolution for species determination, histone H3, translation elongation factor1-α, β-tubulin, actin, and calmodulin have been shown in previous studies to have an adequate number of nucleotide changes to allow species identification. In the present study, we used partial sequences of the histone H3, actin, and calmodulin genes to identify our two isolates as *C. rodmanii*. Our two strains are not specific to water hyacinth, as they are also pathogenic to beet and sugar beet. Similar host ranges were found for *C. rodmanii* strains isolated from Tabasco in México, Zambia, and Brazil, however, the specificity for water hyacinth persists in *Cercospora piaropi* Tharp and *C. rodmanii* Conway, the latter being the most pathogenic.

* *Corresponding author*. Departamento de Biología, División de Ciencias Naturales y Exactas. Campus Guanajuato. Universidad de Guanajuato, Apartado Postal #187, Centro, Guanajuato, Gto, C.P. 36000, México. Tel.: +52 473 732 0006x8159; fax: +52 473 732 0006x8153.
E-mail address: lealc@ugto.mx

1878-6146/$ – see front matter © 2011 British Mycological Society. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.funbio.2011.08.001

Introduction

Cercospora species are among the most prevalent and destructive plant pathogens and are usually found on leaves, pedicels, stems, fruits, and bracts (Beilharz 1994). This group is present on a wide range of hosts, including almost all major families of dicots, most monocot families and even some gymnosperms and ferns (Pollack 1987).

The genus *Cercospora* was described by Fresenius in 1863 (taken from Groenewald *et al.* 2006). Pollack (1987) listed more than 3000 species names, but the list has since been revised, and the number of species was reduced due to synonymy by Crous & Braun (2003), leaving only 659 *Cercospora* species.

Many *Cercospora* species are characterised by the production of a secondary phytotoxic metabolite of polyketide origin called cercosporin (Assante *et al.* 1977), and Fajola (1978) concluded that this trait is diagnostic of true *Cercospora* species. However, the *Cercospora* species concept has also been based upon conidial characteristics and host specificity (Chupp 1954; Ellis 1971), and both criteria have caused confusion. *Cercospora rodmanii* was originally differentiated from *Cercospora piaropi* only by its conidial shape (Conway 1976). Whereas initial molecular characterisation by sequencing of the ITS region, histone H3, translation elongation factor1-α, and β-tubulin suggested these species are the same (Tessmann *et al.* 2001), more recent sequencing of regions of the protein-coding genes calmodulin and another primer set used for histone H3 has suggested that they are actually two distinct species (Groenewald *et al.* 2010). Similarly, although the conidia of *Cercospora apii* are identical to that of *Cercospora beticola*, a multigene phylogenetic comparison and cultural characteristics have shown these to be distinct species with wide and overlapping host ranges (Groenewald *et al.* 2005; 2006).

Various molecular approaches have been used for *Cercospora* identification. Targets for *Cercospora* genus identification have included not only the partial sequences of protein-coding genes histone H3, translation elongation factor1-α, β-tubulin, actin, and calmodulin but also the 18S nrRNA gene and the internal transcribed spacer (ITS) regions that include the gene for 5.8S nrRNA gene (Stewart *et al.* 1999; Tessmann *et al.* 2001; Groenewald *et al.* 2005). The nrRNA genes offer distinct advantages over other molecular targets, including increased sensitivity due to their high copy number per genome and the high conservation level of their sequences. On the other hand, protein-coding genes offer a large number of unlinked sources of phylogenetic information (Geiser *et al.* 1998; Groenewald *et al.* 2010).

Water hyacinth (*Eichhornia crassipes*) is a monocotyledon, free-floating aquatic macrophyte. Its attractive flowers have resulted in its wide distribution by man. The plant reproduces using both sexual and vegetative processes, although the latter is most important (Gopal 1987). Water hyacinth seeds have been reported to survive up to 20 y in a dormant state (Myers *et al.* 1964; Matthews 1967 both cited in Gopal 1987). Water hyacinth has become a serious weed in all freshwater habitats in tropical and subtropical areas, where it interferes with water uses, causes substantial economic hardship and displaces native animal and aquatic plant communities. This weed is able to form giant mats of interconnected plants (Gopal 1987), and it is often referred to as the most costly and troublesome aquatic weed in the world (Holm *et al.* 1991).

Eichhornia crassipes is broadly distributed in tropical regions of Mexico and was probably introduced in early 1900 (Novelo 1996). By 2001, it was estimated that more than 40 000 hectares (ha) of water bodies were infested with the plant (Martínez-Jiménez *et al.* 2001). Martínez-Jiménez & Charudattan (1998) conducted a survey of Mexican native fungi to use for biocontrol of water hyacinth and found that *C. piaropi* and *Acremonium zonatum* could be considered for biological control of the plant in Mexico. In the Yuriria lagoon, which is located in southern Guanajuato state in central Mexico, the water hyacinth has been present since at least 1950 but only became a problem after the lagoon dried up in 1982 (Adán Rivera, fisherman from La Angostura, personal communication). In 2001, our laboratory initiated a survey to isolate fungi able to biocontrol the plant. Twenty-five morphologically distinct fungal cultures were isolated. However, four of them were not identified because spores were not obtained even when 180 different culture conditions were tested (Vieyra-Hernández 2004). Even so, two of the isolates were identified as *Cercospora* species based on a combination of preliminary molecular data, red pigment and colony morphology.

The aim of this study was to identify the two native species of *Cercospora* that were isolated from leaf spots on water hyacinth plants from Yuriria lagoon in Guanajuato, Mexico, using the partial DNA sequences of nrRNA genes and five protein-coding genes (histone H3, translation elongation factor1-α, β-tubulin, actin, and calmodulin). Additionally, we determine the host specificity of our strains and several others collected in Mexico, Zambia, Brazil, and USA.

Materials and methods

Sampling procedure

In this study, during the rainy season in 2001, plants with leaf spots were collected from weed-infested sites of Yuriria lagoon, which is located in southern Guanajuato state at 20° 15′ 0″ N and 101° 5′ 60″ W. The plants were collected and transported to our laboratory in steel tanks filled with water. There, the specimens were dissected and the isolation of pathogens attempted, usually within a week of collection.

Isolation and culture of fungi

Leaf pieces were cut from the margins of necrotic lesions (approximately 9 mm^2). Surface contamination was eliminated by submersion of the plant tissue in a 4 % sodium hypochlorite solution for 2 min, followed by two rinses with sterilised distilled water to remove traces of disinfectant. The leaf fragments were placed on pH 4.5 potato dextrose agar (PDA; Bioxon, Mexico) and incubated at 28 °C. Emergent fungi were isolated, and pure cultures were obtained from hypha tips. Each isolate was labelled with a number to distinguish between isolates originating from the same plant.