PRIMER NOTE

Development and characterization of microsatellite markers for the poplar rust fungi \textit{Melampsora medusae} and \textit{Melampsora larici-populina}

J. STEIMEL,* W. CHEN*† and T. C. HARRINGTON*

*Department of Plant Pathology, Iowa State University, 351 Bessey Hall, Ames, Iowa 50011, †Department of Microbiology and Immunology, Indiana University School of Medicine and Walther Cancer Institute, Indianapolis, IN 46202-5120, USA

Abstract

\textit{Melampsora} species and their hybrids are obligate parasites of \textit{Populus} and \textit{Salix} species worldwide. The increasing interest in \textit{Populus} and \textit{Salix} for biomass and fibre production necessitates genetic markers for population studies of \textit{Melampsora} spp. Libraries enriched for simple sequence repeats were used to develop five microsatellite markers for \textit{Melampsora medusae} and \textit{Melampsora larici-populina}. The variation detected by these markers will be valuable for phylogenetic and population genetic studies, substantiate putative hybrids, and deployment of resistant poplar clones.

Keywords: \textit{Melampsora medusae}, \textit{Melampsora larici-populina}, SSR

Received 20 January 2005; revision accepted 11 February 2005

\textit{Melampsora larici-populina} and \textit{Melampsora medusae} are obligate fungal pathogens that now occur in most parts of the world where \textit{Populus} spp. are grown. \textit{M. larici-populina} is native to Eurasia but has been found in Australia, New Zealand, western United States (Newcombe et al. 2000), and eastern Canada (Innes et al. 2004). \textit{M. medusae} is endemic to eastern United States on \textit{Populus deltoides} but has been introduced to Argentina, Australia, Africa, and western United States. Although interspecific hybrids of fungi are rare or difficult to document, Spiers et al. (1994) and Newcombe et al. (2000) have identified putative hybrids involving these \textit{Melampsora} species. However, markers are not available to genetically confirm these hybrids, and studies using population genetics are not possible. These fungi are obligate parasites, so they cannot be grown in culture, and markers are needed that can be resolved with the DNA extracted from single fruiting bodies (uredinia) of 250 µm diameter or less. Though difficult to identify in fungi relative to plants or animals, the polymerase chain reaction (PCR)-based micro-satellites [simple sequence repeats (SSR)] were the rational choice of markers, and we describe here the characterization of five PCR-based SSR markers for these two \textit{Melampsora} species.

The DNA for microsatellite identification came from urediniospores on inoculated leaves. The isolates were from Ames, Iowa, and Puyallup, Washington. Urediniospores were collected by lightly tapping infected leaves over paper and transferring the collected spores into a micro-centrifuge tube. Total genomic DNA of the urediniospores was extracted using a modified protocol of Hamelin et al. (1996), as described by Chen (2001).

Three microsatellite-enhanced libraries for repeats of CAA, CAG, and CAT were constructed for each species using a modified protocol (Steimel et al. 2004) of Edwards et al. (1996). The enriched DNA samples were run on a 2% agarose gel, and bands or smears that ranged from 300 to 1000 bases were cut out and purified using a GENECLEAN II kit (Bio 101). The DNA was ligated into a pGEM-T easy vector (Promega) using a 1 : 1 insert : vector molecular weight ratio. Ligated plasmids were then transformed into JM109 high efficiency competent cells (Promega) using a 1 : 1 insert : vector molecular weight ratio. The prepared membranes were hybridized with a $^{32}$P-labelled oligonucleotide of the respective repeat (DeScenzo & Harrington 1994) to screen for colonies containing high

Correspondence: T. C. Harrington, Fax: (515)294–9420; E-mail: tcharrin@iastate.edu

© 2005 Blackwell Publishing Ltd
copy numbers of the repeat. The most strongly hybridizing clones were sequenced as described in Steimel et al. (2004).

Primers were designed from the flanking regions of the repeat and tested with the DNA extracted from single uredinia (M. medusae) or from multiple uredinia from an individual Populus leaf (M. larici-populina). The DNA extraction procedure for single uredinia was as described by Hamelin (1996). Amplification was conducted in a 96-well thermal cycler (MJ Research Model PTC-100). For MmCAT-30, amplifications were performed in 20.0 µL with 200 µM dNTP mixture, 4.0 mM MgCl₂, 1× buffer A, 0.3 units Taq DNA polymerase (Promega), 0.2 µM of each primer, and 1 µL of extracted DNA. For MmCAG-11, MmCAA-57, MmCAT-30, and MmCAG101, amplifications were performed in 20.0 µL with 200 µM dNTP mixture, 1× buffer, 0.3 units TaKaRa Ex Taq™ (PanVera), 0.2 µM of each primer, and 1 µL of extracted DNA. The thermal cycler was programmed for an initial denaturation step at 95 °C for 2 min, then 35 cycles at 95 °C for 35 s, 55 °C for 1 min, and 72 °C for 2 min. The HEX-, TET-, and FAM-labelled products were combined (0.5 µL each), electrophoresed on a ABI PRISM 377 DNA sequencer, and the alleles scored to the nearest bp as described in Steimel et al. (2004).

Four polymorphic and one monomorphic loci were identified (Tables 1 and 2) when tested against samples of M. medusae from the United States (Chen 2001) and M. larici-populina collected from Quebec, Canada, and Belgium. Two of the three M. medusae primer pairs did not produce a PCR product with M. larici-populina DNA. Two bands were evident in most samples, indicating heterozygosity in the dikaryotic uredinia. The M. medusae markers were tested across a large collection of single-uredinial pustules of M. medusae collected from Missouri to Minnesota, and these markers were found to be unlinked and in Hardy–Weinberg equilibrium (Chen 2001). Surprisingly, the primers for MmCAG-30 amplified a single, identical band with both species of fungi, in spite of the high number of tandem repeats in this region; this highly conserved, homozygous locus may not be a true microsatellite region.

Although limited in number, three of these five markers proved highly polymorphic and are suitable for detailed population studies of M. medusae (Chen 2001). A fourth marker was highly polymorphic in both species, and there are now at least two loci for the study of M. larici-populina populations. The primers should also be suitable to confirm hybridizations between the two species.

Acknowledgements
Richard Hamelin and Marijke Steenackers kindly provided the DNA samples of Melampsora larici-populina collected from Quebec and Belgium, respectively.

© 2005 Blackwell Publishing Ltd, Molecular Ecology Notes, 5, 484–486
References


