Rev Argent Microbiol. 2015;47(2):167–169

REVISTA ARGENTINA DE MICROBIOLOGÍA

www.elsevier.es/ram

MICROBIOLOGICAL IMAGE

Nuclear acridine orange fluorescence in *Rhizoctonia* isolates from rice

Observación de núcleos de aislamientos de *Rhizoctonia* de arroz por fluorescencia con naranja de acridina

Viviana A. Barrera[a,*], **Susana Gutiérrez**[b], **María A. Cúndom**[b], **Amelia L. Gasoni**[a]

[a] *Instituto de Microbiología y Zoología Agrícola (IMYZA), INTA, Hurlingham, Castelar, Buenos Aires, Argentina*
[b] *Cátedra de Fitopatología, Facultad de Ciencias Agrarias, UNNE, Corrientes, Argentina*

Received 12 December 2014; accepted 26 February 2015
Available online 13 May 2015

The genus *Rhizoctonia* DC (1805) has long been studied as an important soilborne pathogen that causes a wide variety of symptoms because it is a non-specialized pathogen[3]. *Rhizoctonia sensu lato* is characterized by the lack of conidiogenous cells and this taxon is composed of two groups based on the number of nuclei per cell: the multinucleate group that belongs to *Rhizoctonia s. str.* and the binucleate group that belongs to *Ceratorhiza*[5]. Currently, other authors consider the group a *Ceratobasidium–Rhizoctonia* complex[7] and divide it into two groups: BNR (binucleate *Rhizoctonia*-like) and MNR (multinucleate *Rhizoctonia*-like)[9]. Many methods are used to observe the number of nuclei in fungal cells, e.g. safranine O, aniline blue, HCl-Giemsa. Some of these methods apply a staining solution involving laborious, time-consuming procedures that require no equipment (Fig. 1). Other methods use fluorophores, which are rapid and precise[1,2,4,6,8,10].

Since a reliable and rapid method was needed to explore the MNR and BNR groups associated with rice crops in Argentina, we applied an accurate technique to observe the number of nuclei in the strain cells belonging to the *Ceratobasidium–Rhizoctonia* complex.

Small portions of mycelia grown on PDA for 24 h were submerged in 0.01% acridine orange (Sigma–Aldrich, USA) aqueous solution during 10 s. The method applied was a modification of the Yamamoto and Uchida's staining method[11], the latter consisting of veronal buffer. The treated mycelium was observed under epifluorescence light using an OLYMPUS BX 51 microscope (Olympus, Japan). Digital photographs were taken using the Cool Snap-Pro System (Media Cybernetics, USA) (Fig. 2). The nuclei showed green fluorescence and the other cell components orange fluorescence. Thirty isolates from a collection of 36 were multinucleate and 6 were binucleate.

In comparison with the other methods mentioned above, the present method is characterized by easy handling and lower cost.

To our knowledge, this is the first time this methodology has been applied to observe nuclei in cells of *Rhizoctonia* isolates obtained from rice cultivars from Corrientes province, Argentina.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

* Corresponding author.
E-mail address: barrera.viviana@inta.gob.ar (V.A. Barrera).

http://dx.doi.org/10.1016/j.ram.2015.02.005

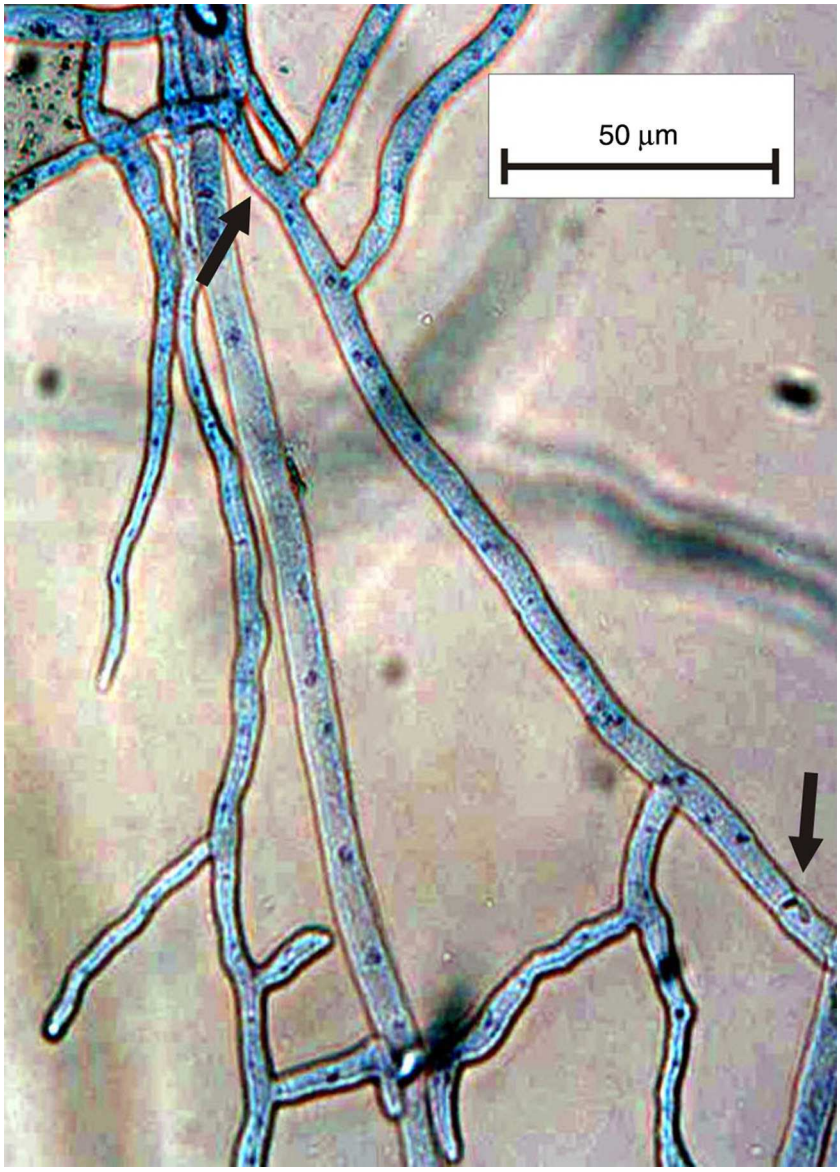

Figure 1 Multinucleate cells stained with lactophenol cotton blue. The arrows show the position of the septa.

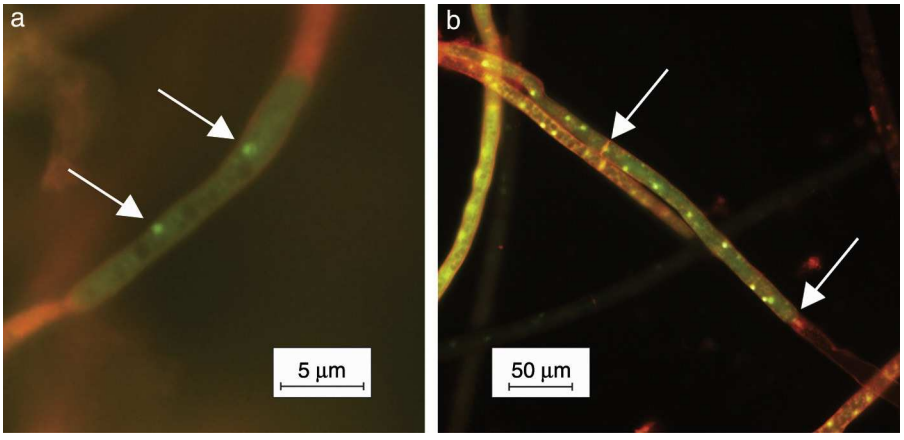

Figure 2 Cells stained with acridine orange: (a) binucleate isolate; (b) multinucleate isolate. The arrows show the nuclei.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Funding, Financial support and acknowledgements

The present research was supported by the Governments of Argentina (Instituto Nacional de Tecnología Agropecuaria) and Japan (Japan International Cooperation Agency) in a bilateral collaboration program.

Conflict of interest

The authors declare that they have no conflicts of interest.

References

1. Cedeño L, Mohali S, Palacios-Prü E. Podredumbre radical causada por *Rhizoctonia solani* en mora de Castilla. Fitopatol Venez. 1994;6:34–7.
2. Cedeño L. Método fácil y confiable para teñir núcleos en hongos del complejo *Rhizoctonia*. Bioagro. 2008;20:215–9.
3. González D, Cubeta M, Vilgalys R. Phylogenetic utility of indels within ribosomal DNA and β-tubulin sequences from fungi in the *Rhizoctonia solani* species complex. Mol Phylogenet Evol. 2006;40:459–70.
4. Kronland WC, Stanghellini ME. Clean slide technique for the observation of anastomosis and nuclear condition of *Rhizoctonia solani*. Phytopathology. 1988;78:820–2.
5. Moore RT. The genera of *Rhizoctonia*-like fungi: *Ascorhizoctonia*, *Ceratorhiza* gen. nov., *Epulorhiza* gen. nov., *Moniliopsis* and *Rhizoctonia*. Mycotaxon. 1987;29:91–9.
6. Oard S, Rush MC, Oard JH. Characterization of antimicrobial peptides against a US strain of the rice pathogen *Rhizoctonia solani*. J Appl Microbiol. 2004;97:169–80.
7. Oberwinkler F, Riess K, Bauer R, Kirschner R, Garnica S. Taxonomic re-evaluation of the *Ceratobasidium–Rhizoctonia* complex and *Rhizoctonia butinii*, a new species attacking spruce. Mycol Prog. 2013;12:763–76.
8. Otero JT, Ackerman JD, Bayman P. Diversity and host specificity of endophytic *Rhizoctonia*-like fungi from tropical orchids. Am J Bot. 2002;89:1852–8.
9. Sharon M, Kuninaga S, Hyakumachi M, Naito S, Sneh B. Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. Mycoscience. 2008;49:93–114.
10. Sharon M, Sneh B, Kuninaga S, Hyakumachi M. The advancing identification and classification of *Rhizoctonia* spp using molecular and biotechnological methods compared with the classical anastomosis grouping. Mycoscience. 2006;47:299–316.
11. Yamamoto DT, Uchida JY. Rapid nuclear staining of *Rhizoctonia solani* and related fungi with acridine orange and with safranin. Mycologia. 1982;74:145–9.