

## Simple New Test for Rapid Differentiation of *Prototheca wickerhamii* from *Prototheca zopfii*

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A simple new test to differentiate *Prototheca wickerhamii* from *Prototheca zopfii* by determining susceptibility to clotrimazole is described. A 50- $\mu$ g clotrimazole disk provides a rapid and reliable means of distinguishing *P. wickerhamii* from *P. zopfii*.

*Prototheca wickerhamii* and *Prototheca zopfii* are two species of achlorophyllous algae that are known to be opportunistic pathogens of humans (3, 4, 5). Separation of the two species is based on macroscopic and microscopic examination and sugar and alcohol assimilation patterns. Immunofluorescence may be used for the confirmation of these two species (6).

In this communication, we describe a possible new differentiating test based on the susceptibility of each species to clotrimazole.

Forty-four strains of *P. wickerhamii*, 10 strains of *Prototheca stagnora* and 21 strains of *P. zopfii* were included in the study. The cultures were received from the collections of L. Ajello, Centers for Disease Control, Atlanta, Ga.; E. H. Ball, University of Glasgow, Scotland; M. Feo, Instituto de Medicina Tropical, Universidad Central de Venezuela, Caracas, Venezuela; P. Hocquet, Service des Maladies Parasitaires et Exotiques, Centre Hospitalier Regional, France; H. Koenig, Institut de Parasitologie et Pathologie Tropical, Université L. Pasteur, Strasbourg, France; C. P. Kurtzman, Agricultural Research Service Culture Collection, Peoria, Ill.; R. S. Pore, Department of Microbiology, West Virginia University, Morgantown, West Virginia; and from our own laboratory (2). The *P. stagnora* strains originated from sewage water, the collections of *P. zopfii* from sewage water, feces, and finger nails, and the *P. wickerhamii* strains from sewage water, skin, and human blood.

Identification of *P. wickerhamii* and *P. zopfii* isolates was confirmed by macroscopic and microscopic examination, and by determining their abilities to use sucrose, trehalose, lactose, inositol, *n*-propanol, and xylose as carbon sources for growth (1). Confirmation of the species identification was made by W. Kaplan, Division of Mycotic Diseases, Centers for Disease Control, by immunofluorescence (6).

The disks used in this study contained 50  $\mu$ g of clotrimazole. Disk diffusion tests were made on 20-ml of Sabouraud glucose agar (Difco Laboratories) in petri (90 mm) dishes inoculated with an even suspension of algal cells. The *Prototheca* inoculum was prepared in distilled water with organisms that were incubated for 7 days at 37°C on Sabouraud glucose agar. The suspensions were adjusted to a no. 1 MacFarland standard before inoculating the plates. Plates were examined on days 2 and 5 after inoculation. All tests were repeated at least twice to confirm reproducibility. Strains were considered resistant if growth was not inhibited by clotrimazole. Inhibition was defined as a zone of inhibition of 10 mm or more.

All strains of *P. zopfii* were resistant to clotrimazole (Table 1). In contrast, all strains of *P. wickerhamii* were susceptible, with an average inhibitory zone diameter of 23 mm.

The results of this study show that clotrimazole can be used for aiding in the separation of *P. wickerhamii* from *P. zopfii*. Susceptibility to this agent is markedly different between these two species. With a concentration of 50  $\mu$ g of clotrimazole per disk, *Prototheca* spp. inhibited at 37°C can be presumed to be *P. wickerhamii*, whereas resistant strains are likely to be *P. zopfii*. All strains of *P. stagnora* tested were susceptible to clotrimazole, but *P. stagnora* does not grow at 37°C.

TABLE 1. Activity of clotrimazole (50  $\mu$ g per disk) against *P. wickerhamii*, *P. zopfii*, and *P. stagnora*

Species	No. of strains tested	No. (%) of strains inhibited	Avg diam (mm) of inhibitory zone	
			Range	Mean
<i>P. wickerhamii</i>	44	44 (100)	10-36	23
<i>P. zopfii</i>	21	0 (0)	0	0
<i>P. stagnora</i>	10	10 (100)	10-30	20

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