

## *Cokeromyces recurvatus*, a Mucoraceous Zygomycete Rarely Isolated in Clinical Laboratories

MAGGI E. KEMNA,<sup>1†</sup> RICHARD C. NERI,<sup>2</sup> RUSSEL ALI,<sup>3</sup> AND IRA F. SALKIN<sup>1\*</sup>

*Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201,<sup>1</sup> and Department of Obstetrics and Gynecology<sup>2</sup> and Center for Laboratory Medicine,<sup>3</sup> Millard Fillmore Hospital, Williamsville, New York 14221*

Received 23 August 1993/Returned for modification 9 November 1993/Accepted 10 December 1993

***Cokeromyces recurvatus* Poitras was isolated from an endocervical specimen obtained from a 37-year-old, insulin-dependent diabetic. The patient's diabetic condition had been well controlled for 10 years, and she had no other known medical problem. This is only the fourth time that this zygomycete has been recovered from a human source. While there was no evidence of tissue invasion in the present patient, the observation of fungus-like structures in two separate Papanicolaou-stained cervical smears prepared 1 year apart suggests that *C. recurvatus* may be capable of colonizing endocervical tissue.**

Zygomycosis is a collective term used to denote opportunistic infections of animals and humans caused by soil saprobes or invertebrate parasites belonging to two orders within the division *Zygomycotina*, i.e., the orders *Mucorales* and *Entomophthorales*. Most human cases of zygomycosis caused by mucoraceous fungi have been associated with metabolic acidosis, immunosuppression, and trauma. Although the types of diseases are quite variable and dependent upon the patient's underlying condition as well as the portal of entry of the fungus, the most common forms of mucoraceous zygomycosis reported in the English literature are rhinocerebral, pulmonary, and systemic infections (6).

*Cokeromyces recurvatus* Poitras is a member of the order *Mucorales* that is usually recovered from the soil or from rodent and lizard dung. It has previously been isolated from three humans and was found to be a colonizer of the vagina and the urinary bladder (3, 4, 7, 8). However, in none of these patients was there evidence of tissue invasion.

We describe in this report a patient from whom *C. recurvatus* was recovered from a vaginal specimen and describe the salient morphologic and physiologic characteristics of the zygomycete.

(Portions of the results described here were presented previously [1a].)

**Case report.** An endocervical specimen was obtained in August 1992 from a 37-year-old, insulin-dependent diabetic as part of a routine gynecological examination. The patient's diabetic condition had been well controlled for 10 years, and her medical history revealed no past or present problems except for an appendectomy and tonsillectomy in childhood. Her family history was equally unrevealing. However, microscopic examination of a routine Papanicolaou-stained (Pap) cervical smear revealed the presence of round, multibudding yeast-like cells morphologically similar to the *in vivo* yeast phase of *Paracoccidioides brasiliensis* de Almeida. Consequently, a second endocervical specimen was collected and was

used to inoculate standard fungal isolation media. The mold recovered from the second specimen was initially identified by the local laboratory as *P. brasiliensis* and was submitted for verification to the Mycology Laboratories of the Wadsworth Center.

These findings precipitated a retrospective examination of the patient's previous Pap smear prepared in 1991. Similar yeast-like cells were observed but were dismissed at the time as vegetative debris.

**Laboratory studies.** A Sabouraud glucose agar (SGA; Difco Laboratories, Detroit, Mich.) culture containing a single, large, light gray mold-like colony and several small, pasty, cream-colored colonies was received by the Mycology Laboratories. A portion of each colony type was mounted in calcifluor white for direct microscopic examination. In addition, portions of the mold-like colony were aseptically subcultured onto SGA slants and were incubated at 30, 37, and 42°C and were also subcultured on potato dextrose agar (PDA; Difco), corn meal agar (Difco), and Mycosel (MYC; BBL/Becton Dickinson Microbiological Systems, Cockeysville, Md.) slants at 30 and 37°C. In contrast, the pasty, yeast-like colonies were subcultured into screw-cap tubes containing brain heart infusion broth (BHI; Difco) plus 0.1% agar (Difco) and slants of yeast extract-peptone-glucose agar (YEPG) (3). The BHI plus 0.1% agar cultures were incubated at 35°C in 5% CO<sub>2</sub> in air, while the YEPG slants were incubated aerobically at 37°C.

Spore ontogeny of the mold was investigated with 7- to 10-day-old PDA slide cultures incubated at 30°C. Thiamine dependency was evaluated with Trichophyton 1 and 4 agar (Difco) slants, which were examined for growth after 10 days of incubation at 37°C. The ability of the mold to utilize potassium nitrate as the sole nitrogen source was studied as described previously (5), while its capability to ferment glucose was determined by the Wickerham broth procedure (2). Sucrose assimilation was assessed with sucrose assimilation agar slants (SAM; Remel, Lenexa, Kans.) after 10 days of incubation at 37°C.

**Microscopic studies.** Microscopic observations of the calcifluor white mounts prepared from the pasty colonies demonstrated the presence of large, thick-walled cells, many bearing multiple buds (Fig. 1). The overall morphology was consistent with the yeast phase of *P. brasiliensis*. In contrast, examination of the microscopic mounts of portions of the mold colony revealed branching, broad, generally nonseptate hyphae as well

\* Corresponding author. Mailing address: Wadsworth Center for Laboratories and Research, New York State Department of Health, P.O. Box 509, Albany, NY 12201-0509. Phone: (518) 474-7413. Fax: (518) 486-5736.

† Present address: Center for Medical Mycology, Department of Dermatology, Case Western Reserve University, Cleveland, OH 44106.

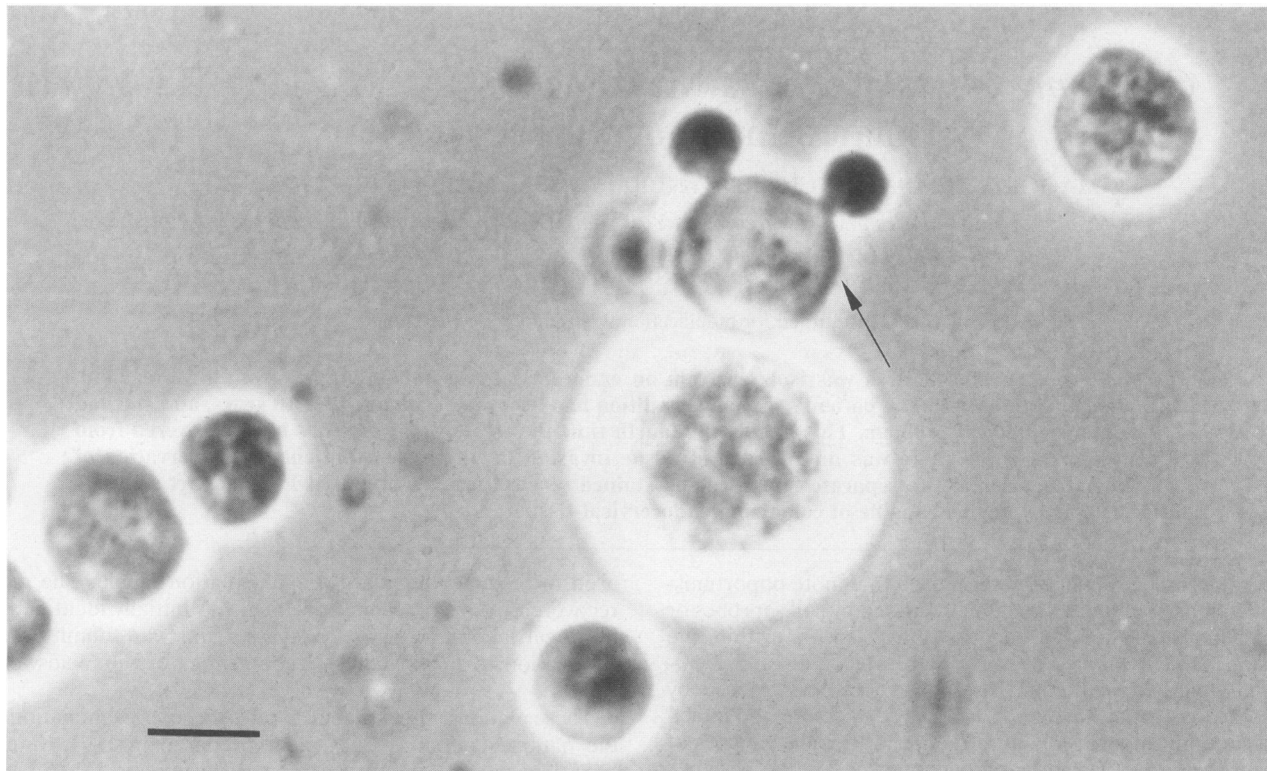


FIG. 1. Thick-walled, budding, yeast-like cells. Note the cell (arrow) with multiple buds. Bar, 20  $\mu\text{m}$ .

as thick-walled, dark brown-black zygospores borne on suspensors (Fig. 2). Consequently, the mold was presumptively identified as a zygomycete.

**Mycology studies.** The initial identification of the mold was confirmed through more detailed studies of the PDA slide cultures. Hyaline, smooth-walled, broad sporangiophores, each

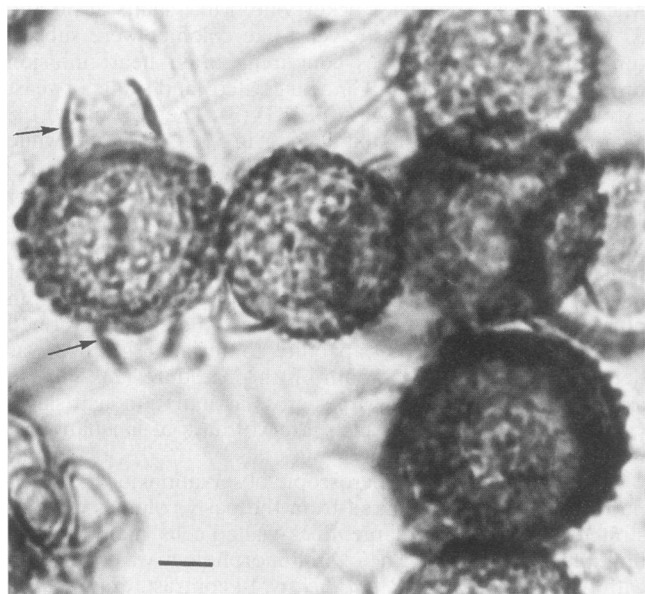


FIG. 2. Zygospores on suspensors (arrows) formed by an isolate grown in culture. Bar, 20  $\mu\text{m}$ .

terminating in a large spherical vesicle, were observed after 10 days of incubation at 30°C. Arising from the vesicles were elongate, recurved stalks or pedicels each bearing globose sporangia approximately 10 to 12  $\mu\text{m}$  in diameter (Fig. 3). In addition, numerous spherical, dark brown-black zygospores borne on suspensors from opposite hyphae were evident in the slide cultures.

Subcultures of the mold grew on all media except MYC at all temperatures; it did not grow on MYC even after 14 days of incubation. While very slow growing and mold-like in appearance at 30°C, far more abundant and rapid development was noted at 37°C. In addition, colonies at the higher temperature were yeast-like in texture and were composed primarily of spherical, thick-walled budding cells. The isolate assimilated potassium nitrate as the sole nitrogen source, but it could not utilize sucrose or ferment glucose as the sole carbon source. Greater growth was noted on Trichophyton 4 than on Trichophyton 1 agar slants.

Subcultures of the yeast-like colonies under both incubation environments were pasty in texture and, as with the mold colonies at elevated temperatures, were chiefly composed of large, spherical cells bearing one to multiple buds.

The observation of few-spored, globose sporangia borne terminally on long recurved pedicels arising from a vesicle at the apex of the sporangiophore and the development of zygospores between opposed suspensors from hyphae on an obviously homothallic mycelium clearly establish the identification of the isolate as *C. recurvatus*.

Rippon and Dolan (7) reported the recovery of a zygomycete from a vaginal specimen. As for the patient described here, the fungus was discovered on a routine Pap smear from a healthy, 30-year-old female. Microscopic examination of the smear revealed the presence of large, thick-walled cells, many

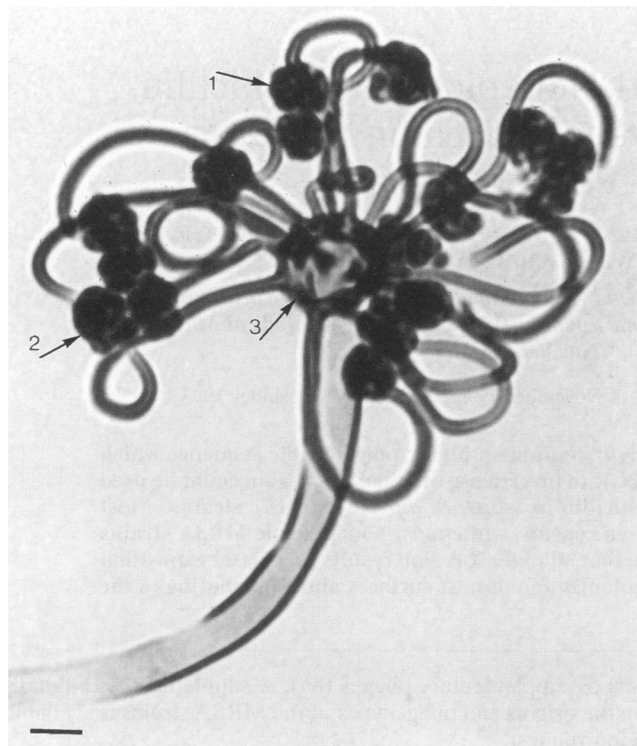


FIG. 3. Sporangia (arrows 1 and 2) formed at the tips of recurved stalks arising from a vesicle (arrow 3). Bar, 20  $\mu$ m.

bearing from one to eight buds. Cultures inoculated with portions of the vaginal specimen yielded a zygomycete originally described as a member of the genus *Mucor* but subsequently reidentified as *C. recurvatus* (8). Since the patient was asymptomatic and the vagina and cervix were found to be normal, the investigators concluded that *C. recurvatus* was only a colonizer or contaminant.

*C. recurvatus* was also isolated in culture by McGough et al. (4) from a 14-year-old primigravid patient with vaginitis. While clinical and mycological cures were achieved by a 14-day course of therapy with an antifungal cream, there was no evidence of tissue invasion, nor were fungal structures observed in the direct examinations of pretreatment vaginal specimens.

Axelrod et al. (1) described the recovery of *C. recurvatus* from urine specimens collected from a 72-year-old male with hemorrhagic cystitis. Large yeast-like cells with one to multiple buds, similar in appearance to *P. brasiliensis*, were again noted

on microscopic observation of the specimens. Since repeated cytologic examinations of the bladder wall did not reveal the presence of the fungus in tissue, the isolation of *C. recurvatus* from multiple urine specimens was attributed to its colonization of bladder debris.

Similarly, in the patient described here, there was no evidence of tissue invasion and no antifungal chemotherapy was instituted. However, the probable presence of *C. recurvatus* in two separate Pap smears prepared 1 year apart suggests that it may be capable of colonizing endocervical tissue.

Kwon-Chung and Bennett reported (3) that *C. recurvatus* converted to a yeast-like form only on YEPD medium at 37°C. In contrast, we found that the isolate from the patient described here would convert from mold-like to yeast-like colonies on the other media that we tested when the colonies were incubated at 35 to 37°C. The physiologic characteristics of the *Cokeromyces* isolate—i.e., growth at incubation temperatures as high as 42°C, an ability to assimilate nitrate as the sole nitrogen source but an inability to utilize sucrose or ferment glucose, thiamine dependency, and susceptibility to cycloheximide—are quite different from those reported for zoopathogenic zygomycetes (9) and could be used, at the generic level, as supplemental identification characteristics.

#### REFERENCES

1. Axelrod, P., K. J. Kwon-Chung, P. Frawley, and H. Rubin. 1987. Chronic cystitis due to *Cokeromyces recurvatus*: a case report. *J. Infect. Dis.* 155:1062-1064.
- 1a. Kemna, M. E., C. R. Cooper, Jr., R. C. Neri, and I. F. Salkin. 1993. Abstr. 93rd Gen. Meet. Am. Soc. Microbiol. 1993, abstr. F36, p. 533. American Society for Microbiology, Washington, D.C.
2. Kreger-van Rij, N. J. W. (ed.). 1984. *The yeasts—a taxonomic study*, 3rd ed. Elsevier Scientific Publications, Amsterdam.
3. Kwon-Chung, K. J., and J. E. Bennett. 1992. *Medical mycology*. Lea & Febiger, Philadelphia.
4. McGough, D. A., A. W. Fothergill, and M. G. Rinaldi. 1990. *Cokeromyces recurvatus* Poitras, a distinctive zygomycete and potential pathogen: criteria for identification. *Clin. Microbiol. Newsl.* 12:113-120.
5. Pincus, D. H., I. F. Salkin, N. J. Hurd, I. L. Levy, and M. E. Kemna. 1988. Modification of potassium nitrate assimilation test for identification of clinically important yeasts. *J. Clin. Microbiol.* 26:366-368.
6. Rippon, J. W. 1988. *Medical mycology: the pathogenic fungi and the pathogenic actinomycetes*, 3rd ed. The W. B. Saunders Co., Philadelphia.
7. Rippon, J. W., and C. T. Dolan. 1979. Colonization of the vagina by fungi of the genus *Mucor*. *Clin. Microbiol. Newsl.* 1:4-5.
8. Rippon, J. W., and C. T. Dolan. 1980. Mucorales: colonization of human vagina and colon, p. 39-42. Proc. VII Congress ISHAM, Jerusalem, 11 to 16 March 1979. *J. Hum. Anim. Mycol.*
9. Scholer, H. J., E. Muller, and M. A. A. Schipper. 1983. Mucorales, p. 9-60. In D. H. Howard (ed.), *Fungi pathogenic for humans and animals*. Marcel Dekker, Inc., New York.