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Use of Paraffin-Embedded Tissue for Identification of *Saccharomyces cerevisiae* in a Baker's Lung Nodule by Fungal PCR and Nucleotide Sequencing

Ping Ren,[1] Sundara Sridhar,[2] and Vishnu Chaturvedi[1,3]*

Mycology Laboratory, Wadsworth Center, New York State Department of Health,[1] and Department of Biomedical Sciences,[3] SUNY School of Public Health, University at Albany, Albany, and Department of Pathology, Coney Island Hospital, Brooklyn,[2] New York

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A 40-year-old healthy male employed in a bakery presented with a single lung nodule and underwent investigations to rule out pulmonary carcinoma. Biopsy was positive for yeast cells, which did not match common fungal pathogens. PCR assay of paraffin-embedded tissue and nucleotide sequencing with ribosomal *ITS1-ITS2* universal primers revealed the presence of *Saccharomyces cerevisiae*.

Identification of fungal pathogens in histological sections frequently requires application of specialized stains (6). Many pathogenic yeasts appear as budding, rounded cells without any characteristic tissue forms (9). This situation is alleviated in instances in which the incriminating fungus can be isolated in culture. However, tissue specimens are not always available for culture. Recently, the application of PCR and nucleotide sequencing has been extended for identification of pathogenic fungi in histological sections. The paraffin-embedded tissue is used as a source of template DNA for a PCR assay with universal fungal ribosomal gene primers and/or a nested PCR assay with pathogen-specific primers, and the amplicons are then analyzed by restriction fragment length polymorphism and/or nucleotide sequencing for confirmation of fungal identity (2–5, 8, 11, 13). This approach is very promising in diagnostics, as it could lead to conclusive identification of the causal pathogen independently of histological or culture observations. We describe a case of a lung nodule in a healthy male that proved to be histologically negative for suspected lung carcinoma and instead revealed budding yeast cells, which were confirmed as *Saccharomyces cerevisiae* by PCR and nucleotide sequencing.

A 40-year-old healthy male was referred to the surgeon at Coney Island Hospital for a lung nodule discovered during a routine chest X-ray done as part of an annual physical examination. The patient was a nonsmoker with no history of any medical illness. A wedge resection of the lung was performed. A 0.7-cm-diameter solid grey-tan nodule was present in the lung parenchyma. The edges of the lesion were sharply demarcated from the surrounding normal lung parenchyma without any calcification. Histopathologic examination revealed an inflammatory mass composed of a background of fibrotic tissue with a moderately dense population of inflammatory cells composed of an equal admixture of histiocytes and lymphocytes. No discrete areas of necrosis were noted. Numerous oval-to-spherical structures reminiscent of fungal cells were revealed by hematoxylin-and-eosin staining (Fig. 1A). A silver methenamine stain confirmed that these were fungal cells, variable in size from 5 to 15 μm, mostly extracellular with occasional budding (Fig. 1B). Since the tissue was not saved for fungal culture, identification of the cells was first attempted on the basis of their morphology. The usual pathogenic fungi causing pulmonary nodular lesions are *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Cryptococcus neoformans*. The larger size and predominant extracellular location were atypical for *H. capsulatum*, the absence of broad-based budding ruled out *B. dermatitidis*, and the absence of a mucinous capsule ruled out *C. neoformans*. Also, the size and morphology were inconsistent with *Coccidioides* species. *Paracoccidiodes brasiliensis* was also a consideration because of travel to South America; however, the morphology was not supportive. The presence of budding cells ruled out the possibility of *Pneumocystis carinii*. No other microorganisms were identified in the lesion.

The specimen was submitted to the Mycology Laboratory at the Wadsworth Center for further investigations. The lung tissue block was sliced into thin pieces with a sterile surgical blade and transferred to a microcentrifuge tube. A 200-μl aliquot of xylene (Sigma, St. Louis, Mo.) was added, mixed by inversion, heated for 15 min at 37°C, and centrifuged at 14,000 rpm in an Eppendorf model 5415 D centrifuge for 15 min. The supernatant was removed, a fresh 200-μl aliquot of xylene was added, and the whole step was repeated once. The pellet was washed twice with 1.0 ml of 100% ethanol for 30 min at 37°C to remove residual xylene. The ethanol was removed by centrifugation for 15 min, and the tissue pellet was air dried for DNA extraction. The QIAamp DNA Mini Kit (Qiagen, Valencia, Calif.) was used to extract the DNA from the tissue pellet in accordance with the manufacturer's protocol. PCR was performed with universal primers designed to amplify the *ITS1* region of the fungal ribosomal DNA. The oligonucleotide sequences were 5′-TCCGTAGGTGAACCTGCGG-3′ and 5′-GCTGCGTTCTTCATCGATGC-3′ (12). The thermal cycling conditions were initial denaturation at 95°C for 5 min, followed

* Corresponding author. Mailing address: Mycology Laboratory, Wadsworth Center, New York State Department of Health, SUNY School of Public Health, University at Albany, Albany, NY 12201-2002. Phone: (518) 453-6412. Fax: (518) 486-7811. E-mail: vishnu@wadsworth.org.

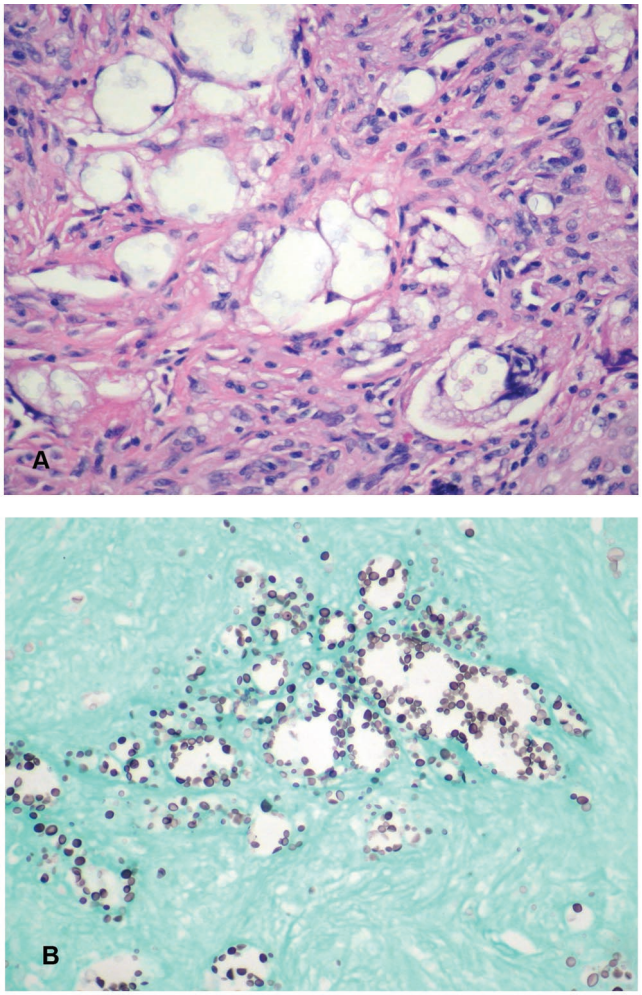

FIG. 1. Histological examination of lung nodule. (A) Hematoxylin-and-eosin staining revealing budding round-to-oval structures in the midst of inflammatory cells (lymphocytes and plasma cells). Original magnification, ×400. (B) Silver methenamine staining revealing extracellular fungal cells with occasional budding. Original magnification, ×400.

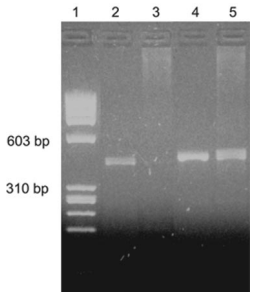

FIG. 2. Electrophoresis of DNA amplified by PCR of lung nodule. Lanes: 1, φX174 RF/HaeII DNA fragment used as a size standard; 2, positive PCR result with lung nodule DNA amplified by fungal *ITS1* primers; 3, negative PCR result with control human DNA and fungal *ITS1* primers; 4 and 5, positive PCR results with DNA from lung nodule and control human DNA amplified with primers for the human androgen receptor.

by 35 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 30 s, and extension at 72°C for 1 min and a final extension step of 72°C for 10 min. The same reaction conditions were subsequently used to amplify the *ITS1-ITS2* regions with the primers 5′-TCCGTAGGTGAACCTGCGG-3′ and 5′-TCCTCCGCTTATTGATATGC-3′ (12). The DNA template was also used for PCR amplification of the human androgen receptor gene as a control with primers AR-F (5′-GCCTGTTGAACTCTTCTGAGC-3′) and AR-R (5′-GCTGTGAAGGTTGCTGTTCCTC-3′) (10). The control human DNA sample was prepared from blood. The PCR products were electrophoresed on 1.2% agarose gel in Tris-borate-EDTA buffer. The DNA amplicons were purified from the gel with a QIAquick gel extraction kit (Qiagen) and sequenced with an ABI PRISM 377 sequencer and a BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, Calif.).

The template DNA was successfully extracted from the paraffin-embedded lung tissue, and PCRs worked well with both fungal and human primers (Fig. 2). One prominent DNA band of approximately 400 bp was obtained with *ITS1* universal fungal primers and not with the control human DNA (lanes 2 and 3), which indicated that only the lung nodule sample had fungal DNA. The positive bands of approximately 475 bp in lanes 4 and 5 were obtained with human androgen receptor primers from both lung nodule DNA and control human DNA, which indicated that the extracted DNA was comparable in quality to control human DNA prepared in the laboratory. Nucleotide sequencing of the DNA amplicon from lane 2 and homology searches in the GenBank database revealed the highest homology with the corresponding *S. cerevisiae* sequences. The subsequent sequencing of the 840-bp *ITS1-ITS2* fragment from lung nodule DNA also revealed the highest homology with *S. cerevisiae*.

It is reasonable to suggest that the lung nodule in this patient was caused by inhalation of dry baking yeast powder since the patient must have been exposed during his work, which involved setting up bakeries in different areas. The patient did not receive any further treatment after surgery, and additional blood tests did not reveal any immune dysfunction. The patient is doing well and has been disease free for 2 years. Although *S. cerevisiae* is a rare fungal pathogen, mostly in immunocompromised patients, sporadic cases of vaginitis and asthma have been attributed to occupational exposure (1, 7, 14).

A number of investigators have described the use of a nested-PCR approach combining *ITS* universal primers with species-specific primers to confirm the etiology of *B. dermatitidis* and *H. capsulatum* in paraffin-embedded tissue (2, 3, 8, 11). This approach increases the specificity of the PCR and obviates the need for subsequent sequencing to confirm identity, but it cannot be used with samples containing unknown fungi. Two other groups have reported detailed comparisons of PCR with *ITS* primers, followed by hybridization with species-specific nucleic acid probes and/or nucleotide sequencing (5, 13). In one of these studies, it was found that hybridization with specific probes was rather limited in its sensitivity and specificity compared to nucleotide sequencing (13). In conclusion, the present report highlights the pathogenic potential of *S. cerevisiae* in healthy humans who may acquire the fungus by

occupational exposure. Our laboratory findings also confirm that the combination of PCR and nucleotide sequencing with *ITS1-ITS2* primers is a viable option for the identification of fungal pathogens from paraffin-embedded tissue, especially when the identity of the fungus is unknown.

Nucleotide sequence accession numbers. The sequences determined in this study have been deposited in the GenBank database with accession numbers AF500488 and AY525600.

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