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Specificity of Exoantigens for Identifying Cultures of *Coccidioides immitis*

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Forty-nine isolates of arthroconidial fungi other than *Coccidioides immitis* did not produce immunodiffusion lines of identity with the reference system for that fungus.

Standard and Kaufman (6, 7) have reported that soluble antigens (exoantigens) in concentrated-broth culture filtrates could be used in immunodiffusion tests for rapid identification of *Histoplasma capsulatum* and *Coccidioides immitis*. The accuracy of this method depended on whether precipitated bands of identity were produced with an established reference system of antigen and antiserum for histoplasmosis and for coccidioidomycosis, respectively. The H and M bands, originally reported by Heiner (1), were considered specific for *H. capsulatum*. A total of 96 unknown cultures were tested, and 41 of the 48 developing bands of identity proved to be *H. capsulatum* subsp. *capsulatum*, whereas 7 were identified as *H. capsulatum* subsp. *duboisii*. The remaining 48 were species other than members of the genus *Histoplasma*; exoantigens from 5 were reactive but nonidentical, and the remaining 43 were negative. The coccidioidin-anticoccidioidin reference system developed three bands believed to be specific for *C. immitis*. Two were the immunodiffusion correlating with complement fixation (IDCF) and immunodiffusion correlating with tube precipitation (IDTP) reactions reported originally by Huppert et al. (3), and the third was designated immunodiffusion heat-labile antigen (IDHL) by Standard and Kaufman (7). This test was applied to 166 unknown fungus cultures; exoantigens from 66 reacted with one or more bands of identity and proved to be *C. immitis* by conventional tests. Three of the remaining 100 preparations developed one band of nonidentity, 97 were nonreactive, and all 100 were from species other than *C. immitis*. Therefore, these studies supported the interpretation that bands in the reference systems for *H. capsulatum* and *C. immitis*, respectively, are specific for these fungi.

Since *C. immitis* is only one of the many fungi with thallic arthroconidia (5), we have investigated the specificity of these exoantigens against 49 cultures of fungi resembling *C. immitis* (Table 1). The method was described by Standard and Kaufman (6, 7) except that we used a larger immunodiffusion pattern (2). Coccidioidin and anticoccidioidin for the reference system were supplied by L. Kaufman, and, even though coccidioidins prepared by us yielded complete identity with this reference system, the coccidioidin furnished by Kaufman was used in this study to reproduce accurately the test as reported by Standard and Kaufman (6, 7). Exoantigens from three cultures gave bands of nonidentity: *Auxarthron umbrinum* UAMH 3952, *Geotrichum* sp. UAMH 2098, and *Uncinocarpus reesii* UAMH 1706. All other preparations were nonreactive. In addition, we demonstrated at least one exoantigen of identity in each of more than 100 isolates of *C. immitis*, including many with atypical characteristics (4) and one diauxotrophic mutant, no. 95-271 from Henry Walch (9). The fungi tested by Standard and Kaufman had included 19 isolates with arthroconidia, and, combined with the 49 in our series, a total of 68 fungus cultures resembling *C. immitis* did not produce reactions of identity with the coccidioidin-anticoccidioidin reference system. The importance of the appropriate reference system is emphasized by the nonidentity reactions from six isolates in the two series.

Even though the evidence is very strong for species specificity of the IDCF, IDHL, and IDTP bands, we hesitate to rely upon this alone as an absolute identification of *C. immitis*. There are still many arthroconidial fungi that have not been tested. Since some of those studied to date have produced nonidentical antigens reacting with the reference system antiserum, it is still conceivable that examination of additional strains might demonstrate that one or more of the three bands in the reference system is not specific for *C. immitis*. In addition, we encountered one stock culture isolate of *C. immitis* which, for unknown reasons, grew poorly in brain heart infusion broth. The supernatant

TABLE 1. *Arthroconidia-producing fungi tested for exoantigens found in the reference system for C. immitis*

Fungus	No. of strains	Strain No.[a]
Amauroascus niger	1	UAMH 3544
A. verrucosus	1	UAMH 3563, ATCC 18744 (as *Arachniotus verrucosus*)
Arachniotus reticulotus	1	CDC B-740
Arcuadendron ovatum	1	UAMH 2737
Arthrographis langeroni	2	UAMH 3380; UAMH 3616c
Auxarthron californiense	1	UAMH 3151, NRRL A-11
A. conjugatum	1	UAMH 3156
A. reticulatun	1	UAMH 3154, NRLL A-10, 748
A. thaxteri	1	UAMH 3912, ATCC 15598, NRRL 1714
A. umbrinum	3	UAMH 1117; CDC B-737; UAMH 3952, ATCC 15606; NRRL 3657
A. zuffianum	1	UAMH 1875, ATCC 13484
Geotrichum sp.	2	UAMH 1537; UAMN 2098
Gymnoascus brevisetosus	1	UAMH 3079
G. uncinatus	2	UAMH 1584; UAMH 3913, NRRL 3610
Malbranchea albolutea	1	UAMH 2846
M. arcuata	1	UAMH 1861
M. aurantiaca	3	UAMH 1709; UAMH 1778; UAMH 3599
M. chrysosporoides	3	UAMH 1031; UAMH 2786; UAMH 1060
M. circinata	1	UAMH 1890
M. dendritica	2	UAMH 3953; UAMH 2731
M. flava	2	UAMH 1589; UAMN 1879
M. flavorosea	1	UAMH 1065
M. fulva	3	UAMH 2851; UAMH 3901; UAMH 1050
M. gypsea	4	UAMH 1975; UAMH 1841; UAMH 1842; UAMH 1843
M. sulfurea	1	UAMH 3761
Myxotrichum sp.	1	CDC B-739
M. carminoparum	1	UAMH 1597
M. stipilatum	1	UAMH 1510
Pseudoarachniotus sp.	1	CDC B-738
P. hyalinosporus	1	UAMH 3155; NRRL 2881
Uncinocarpus reesii	3	UAMH 1704; UAMH 1706; UAMH 1273

yielded a negative test for *C. immitis* with the routine 10× and 25× concentrations, but a repeat test with a 50× concentration was positive. If this culture had been a primary isolate, it might have been reported falsely negative for *C. immitis*. Therefore, those who adopt this procedure should be warned that, when poor growth occurs in the broth, either incubation should be continued beyond 3 days or the supernatant should be concentrated to 25× and 50×.

There are advantages to having an additional, apparently reliable, test for identifying a culture as *C. immitis*. Standard and Kaufman (7) commented about the usefulness of the exoantigen test for identifying nonsporulating cultures, although in our experience this has been a rather rare occurrence among literally hundreds of primary isolates of *C. immitis*. A second advantage derives from our experience. Even though we have prided ourselves on the care and accuracy with which we established identification procedures for *C. immitis*, we have made this identification incorrectly for at least one culture, and this was detected by Kaufman with the exoantigen test among a group of cultures sent to him as atypical *C. immitis*. Since then, we have reconfirmed our identifications of other "atypical" cultures of *C. immitis* (4) by the exoantigen test, by in vitro conversion of arthroconidia to endosporulating spherules (8), and by animal inoculation procedures. Sigler and Carmichael commented that we had "apparently confused some isolates of other *Malbranchea* species with *C. immitis*" even though they produced coccidioidomycosis in mice, and they pointed out that we had made "no mention of controls to rule out the possibility of accidental infection of their mice with *C. immitis* (5)". Their point was most relevant because we had not mentioned that, in fact, we routinely had performed air sampling and had sacrificed "monitor" mice for this purpose. As noted above these identifications have been reconfirmed with three procedures. The relevant point is that many arthroconidial cultures can be confused with *C. immitis* and vice versa. Hence, we consider the exoantigen test to be a useful and valuable aid for identifying isolates as *C. immitis*. Nevertheless, we continue to recognize that practical experience with this new procedure is still very limited, and the potential for reporting false positive or false negative results requires additional experience. It is our

[a] UAMH, University of Alberta Mold Herbarium and Culture Collection, Edmonton, Canada; CDC, Center for Disease Control, Atlanta, Ga.; NRRL, Northern Utilization Research Development Division, Peoria, Ill.

opinion, admittedly conservative but we believe justified, that a positive exoantigen test justifies early reporting of a presumptive identification as *C. immitis* to be confirmed by conventional procedures.

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LITERATURE CITED

1. **Heiner, D. C.** 1958. Diagnosis of histoplasmosis using precipitin reactions in agar gel. Pediatrics **22**:616–627.
2. **Huppert, M., and J. W. Bailey.** 1965. The use of immunodiffusion tests in coccidioidomycosis. I. The accuracy and reproducibility of the immunodiffusion test which correlates with complement fixation. Am. J. Clin. Pathol. **44**:364–368.
3. **Huppert, M., J. W. Bailey, and P. Chitjian.** 1967. Immunodiffusion as a substitute for complement fixation and tube precipitin tests in coccidioidomycosis, p. 221–225. *In* L. Ajello (ed.), Coccidioidomycosis. University of Arizona Press, Tucson.
4. **Huppert, M., S. H. Sun, and J. W. Bailey.** 1967. Natural variability in *Coccidioides immitis*, p. 323–328. *In* L. Ajello (ed.), Coccidioidomycosis. University of Arizona Press, Tucson.
5. **Sigler, L., and J. W. Carmichael.** 1976. Taxonomy of *Malbranchea* and some other hyphomycetes with arthroconidia. Mycotaxon **4**:349–488.
6. **Standard, P. G., and L. Kaufman.** 1976. Specific immunological test for rapid identification of members of the genus *Histoplasma*. J. Clin. Microbiol. **3**:191–199.
7. **Standard, P. G., and L. Kaufman.** 1977. Immunological procedure for the rapid and specific identification of *Coccidioides immitis* cultures. J. Clin. Microbiol. **5**:149–153.
8. **Sun, S. H., M. Huppert, and K. R. Vukovich.** 1976. Rapid in vitro conversion and identification of *Coccidioides immitis*. J. Clin. Microbiol. **3**:186–190.
9. **Walch, H. A., and R. K. Walch.** 1967. Studies with induced mutants of *Coccidioides immitis*, p. 339–347. *In* L. Ajello (ed.), Coccidioidomycosis. University of Arizona Press, Tucson.