Microbiological Testing of Aerosol Preparations Using *Chaetomium globosum*

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Chaetomium globosum, **a common fungus of the soil, and a member of the class *Ascomycetes,* is a cellulolytic organism, whose growth is dependent primarily upon the degradation of cellulose material. This study explores the possibility of utilizing a modified growth and test procedure applicable to the A.O.A.C. recommended prescribed method for testing antifungal activity of aerosols. The test procedure developed brings the method to the organism under conditions that are applicable to the natural growth habits. A convenient method is described employing the inhibition of growth of *C. globosum* on sterile filter paper after exposure to certain aerosols. Sterile phenol solutions and certain blends of quaternary ammonium compounds were used as the challenging agents.**

THE USE of aerosol preparations in the treatment of fungal infestations requires appropriate microbiological testing procedures. At present, the A.O.A.C. (1) employs a method involving a *Trichophyton* sp., a pathogen, as the test object. In these laboratories, a simplified, convenient, and reliable method has been developed utilizing a nonpathogenic fungal organism, *C. globosum*. *C. globosum* (ATCC-6205) a common fungus of the soil, is a cellulolytic organism, depending primarily upon the degradation of cellulose materials for its growth (2). A sterile strip of filter paper was placed on a cooled tube-slant of a suitable medium,[1] and the strip was inoculated with spores. This was used as the stock culture after incubation for 7 days at 25–30°. Sterile Whatman No. 2 filter paper was used as the source of cellulose. This growth pattern and property was adapted to the evaluation of antifungal activity, since the inhibition of growth of this organism on sterile filter paper was easily observed after exposure to certain aerosols.

PROCEDURE

The A.O.A.C. spray test procedure (1, 3, 4) for evaluating the antifungal activity of aerosol preparations recommends that a standardized spore suspension of *Trichophyton interdigitale* (ATCC-640) be spread over an area of 1 sq. cm. Thus, it was decided that the test organism for this study would be the spores and vegetative structures of *C. globosum*. This stage of growth was produced on 1-cm. squares of filter paper at the end of 7 days at 25–30°. Spray testing (1) was performed at this stage of growth.

A 1-cm. square containing the test organism was removed from the medium, using sterile forceps, and placed on a sterile glass slide in a Petri dish, the bottom of which was layered with two disks of sterile filter paper. This was repeated for all of the remaining squares (10 per test). The above were placed in an incubator at 25–30° for 2 hr. to allow the squares to dry.

At the end of this period, the plate was supported at a 45° angle, and the respective squares were sprayed using phenol controls and test compounds (Table I) under test for the specified period of time,[2] at a distance of 8 to 10 in. from its surface. The above procedure was repeated for all plates under test. A DeVilbiss No. 251 atomizer was used to produce the spray of the phenol control solutions.

After all squares were sprayed, each one was aseptically removed, respectively, from the glass slide, and was placed on the filter paper bed Petri dish. The dishes were placed in an incubator 24 hr. for drying the sprayed filter squares.

Using aseptic technique, a 2 × 2-cm. square of sterile filter paper was placed on the medium in a Petri dish and the dried 1-cm. square was transferred on to the 2 × 2-cm. square. This was repeated for all the recommended number (ten) of 1-cm. squares. All of the above dishes were incubated for 7 days at 25–30°. Untreated 1-cm. squares containing the viable test organism, were transferred to 2 × 2-cm. squares to serve as controls. This insures the viability of the test organism on both the 1 and 2 × 2-cm. squares and serves to establish a base for the growth pattern of the organism. Growth of the test organism on to the 2 × 2-cm. square at the end of the 7 days was read as positive, while no growth was read as negative (Tables II and III).

The 1 and 2 × 2-cm. squares displaying no apparent growth 7 days after spraying were divided into two groups of equal number. Control procedures were initiated on one group immediately, while the other group was reincubated for an additional 7 days. The same control procedures were then repeated on this second group. (Those squares exhibiting no growth after the first 7 days of incubation remained so for the additional 7 days.)

The following control procedures were utilized for the negative no-growth squares.

A.—The 1-cm. square was removed from the original backup square, inverted on a new 2 × 2-cm. square on fresh medium, and reinoculated with spores from a stock culture. The above was repeated until half of the 1-cm. squares were treated.

B.—The remaining 1-cm. squares were inverted

Received April 25, 1966, from the Research Laboratories, College of Pharmacy, Rutgers—The State University, Newark, N. J. 07104.

Accepted for publication August 16, 1966.

Presented to the Pharmaceutical Technology Section, A.PH.A. Academy of Pharmaceutical Sciences, Dallas meeting, April 1966.

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[1] Medium recommended by American Type Culture Collection: $NaNO_3$, 2.0 Gm., $MgSO_4$, 500 mg., KCl, 500 mg., KH_2PO_4, 140 mg., K_2HPO_4, 1.20 Gm., yeast extract, 20 mg., agar, 15.0 Gm., water, 1.0 L., $Fe_2(SO_4)_3 \cdot XH_2O$, 10 mg. The final pH of the medium is in the range 7.0–7.2.

[2] Spraying time with the DeVilbiss No. 251 atomizer was 5 sec., while spraying time with the commercial pressurized aerosols (as with the quats) was 3 sec.

TABLE I.—SOLUTIONS AND COMPOUNDS TESTED

1, Sterile phenol solutions of the following concentrations (% w/v): 0.5; 1.0; 2.0; 3.0; 4.0; 5.0; 5.25; 5.75; 6.0; 7.0.	
2, Pressurized aerosols of quaternary ammonium compounds. The two formulations used were as follows (% w/w):	
A, BTC-2125[a]	0.1
Dichlorodifluoromethane[b]	50.0
95% Ethanol	49.9
B, Cyncal-type 14[c]	0.1
Dichlorodifluoromethane[b]	50.0
95% Ethanol	49.9

[a] Onyx Chemical Corp., Jersey City, N. J. *n*-Alkyl dimethyl benzyl ammonium chlorides, 25% (C_{14}, 60%; C_{16}, 30%; C_{12}, 5%; C_{18}, 5%). *n*-Alkyl dimethyl ethylbenzyl ammonium chlorides, 25% (C_{12}, 50%; C_{14}, 30%; C_{16}, 17%; C_{18}, 3%). Inert, 50%. [b] Marketed as Freon-12 by E. I. du Pont de Nemours & Co., Wilmington, Del. [c] Sterwin Chemicals, Inc., Industrial Chemicals Division, Subsidiary Sterling Drug, Inc., New York, N. Y. Alkyl dimethyl benzyl ammonium chlorides, 80% (C_{14}, 50%; C_{12}, 40%; C_{16}, 10%). Ethanol, 20%.

TABLE II.—STANDARDIZING PHENOL ACTIVITY USING *C. globosum* AS TEST ORGANISM

% Phenol (% w/v)	Sq. Showing Growth 7 Days After Spraying (%)
Untreated controls	100
0.5	100
1.0	100
2.0	100
3.0	100
4.0	100
5.0	100
5.25	60
5.50	30
5.75	10
6.0	0
7.0	0

TABLE III.—ANTIFUNGAL ACTIVITY OF TWO PRESSURIZED AEROSOL FORMULATIONS

Formulation	Sq. Showing Growth 7 Days After Spraying (%)
A, BTC-2125[a]	0
B, Cyncal-type 14[a]	0
Untreated controls	100

[a] See Table I.

directly on fresh medium and reinoculated with spores from a stock culture.

C.—All 2 × 2-cm. backup squares, on the original medium, were inoculated directly with spores from a stock culture.

Controls were incubated for 7 days. Growth of the organism in each of the controls at the end of this period indicates antifungal activity of the test material, and could not be due to stasis caused by possible absorption of the test material by the filter paper.

In addition, an uninoculated square was sprayed,[3] according to the prescribed procedure, and placed in an incubator for 24 hr. It was transferred to a Petri dish and challenged with spores from a stock culture.

All stasis controls displayed growth in 7 days.

CONCLUSIONS

The results presented in this paper suggest the possiblity of utilizing the prescribed procedure for testing the antifungal activity of certain aerosol preparations using *C. globosum*. Similar organisms could be adapted to the test method. Since the only challenging chemical employed was phenol, and the problem of differentiating between stasis and cidal effects in any procedure of this kind was complicated by the substantive properties of the chemical and its ability to diffuse into the media and/or the back-up 2 × 2-cm. squares in this instance, it would appear that differentiation between stasis and cidal activity is valid.

The test organism, used throughout the procedure, consisted of both the spores and the entire vegetative structure. It will be recognized, however, that if the material under test was effective against spores, it should be similarly effective against the vegetative cells. It could be assumed that the use of the test material on the entire structure of the organism was a more drastic test and closely simulates the use of the test material under the natural growth conditions.

The use of the 1-cm. square of filter paper for the growth of the test organism and its use in the aerosol testing procedure was considered to be valid, since the spores inoculated on any given square were under the exact same growth conditions, with respect to time, temperature, and medium, as those on other squares. This was further substantiated by the fact that the growth of the organism was restricted to the 1 sq. cm. area of the filter paper. Thus, it could be assumed that any given 1-cm. square, at the end of 7 days, contains approximately the same amount of vegetative growth and about the same number of spores.

Experience in these laboratories indicate that the procedure described is simpler, more convenient, more reliable, and safer than the present A.O.A.C. method.

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[3] 6.0% phenol, BTC-2125, and Cyncal-type 14 were the three test compounds used in this particular control.