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Isolation of *Histoplasma capsulatum* from Soil by Direct Culture Methods. (28964)

COY D. SMITH AND ROBERT J. WEEKS (Introduced by Michael L. Furcolow)
Communicable Disease Center, Public Health Service, U. S. Department of HEW, Kansas City Field Station, Kansas City, Kans.

The isolation of *Histoplasma capsulatum* from the soil is a fairly common practice. The methods used by many investigators(1-3) are modifications of the flotation technique described by Stewart and Meyer(4) used to isolate *Coccidioides immitis* from the soil. These methods involve injection of mice with the test material to isolate *H. capsulatum*. Only one report is available in which investigators used another method of isolating the organism from soil without the use of animals. This was one isolation by direct culture obtained by Furcolow and Larsh(5).

This paper reports the use of direct culture to isolate *H. capsulatum* from 2 separate soil specimens.

Methods. Two soil specimens, labeled #1, and #2, were collected from a starling (*Sturnis vulgaris*) roost, in one gallon ice cream cartons. Immediately upon arrival in the laboratory the 2 specimens were tested by an indirect mouse inoculation technique modified from the procedure previously described by Larsh(2). Six weeks later *H. capsulatum* was cultured from the mouse tissues. During this 6 weeks period the remainder of the 2 soil specimens was stored at room temperature in the closed collection cartons. The specimens were still slightly moist after 6 weeks of storage, and various kinds of fungi were visible growing on the surfaces.

When the 2 soils were found to contain *H. capsulatum* by the indirect method, a direct culture method was tried. The medium used to culture the soil suspensions was recently described by Smith(6). The concentration of yeast extract used was 6.0 ml of a 15% stock solution per liter of medium. In addition, antibiotics were added in the following concentrations: penicillin 20,000 units, streptomycin 40 mg, polymyxin B 10 mg, and cycloheximide 1.0 g per liter of medium. All of the above ingredients were added to the warm 2% agar suspension after it was autoclaved. The pH of the medium was 6.0.

Ten grams of each soil specimen were diluted 1:10 by placing into a 250 ml stoppered Erlenmeyer flask containing 90 ml of sterile distilled water. The soil suspensions were mixed thoroughly by hand shaking and further dilutions of 1:100 and 1:1000 were made in sterile distilled water using serial dilution techniques. One-tenth ml of the 1:100 dilution of the suspension of soil #1 was inoculated onto 40 plates of the media described above. The inoculum was spread evenly over the surface using curved glass

rod spreaders. In the same manner, the 1:1000 dilution of the same suspension was inoculated onto 80 plates of medium. The 1:1000 dilution of the suspension of soil #2 was cultured on 60 plates. All plates were incubated for one month in a 27°C water jacket incubator.

Results. It was obvious after 2 weeks incubation that many of the plates had colonies that appeared to be *H. capsulatum* based on microscopic examination. When the one month incubation period had passed, all plates were examined carefully and mounts were made of the suspicious colonies for microscopic examination. Identification of the colonies was based chiefly on the typical morphology of the tuberculated macroconidia. However, many of the colonies were transferred to new media for isolation, then onto brain heart infusion blood medium where they readily converted to the yeast phase when incubated at 37°C. Also, several of these suspicious colonies were injected into mice; and, one month later, typical *H. capsulatum* was cultured from liver and spleen tissues. The colonies which proved to be *H. capsulatum* were tan in color and were diffuse especially around the periphery. One could see by microscopic examination small thread-like individual hyphae bearing microconidia and tuberculated macroconidia as seen in Fig. 1. The number of plates used for each dilution, the number of colonies of *H. capsulatum* isolated from each soil specimen, and the estimated number of organisms per gram of soil are shown in Table I. Many of the plates did not have any colonies of *H. capsulatum,* others contained as many as 5-6 colonies. This inconsistency was true also for the colonies of other fungi present on the plates. Although numerous colonies of saprophytic fungi were present, overgrowth of the plates did not create a problem. No colonies of bacteria were seen on any of the culture plates.

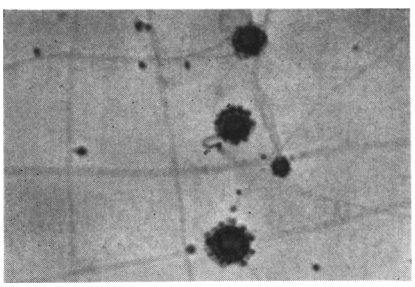

FIG. 1. *Histoplasma capsulatum* isolated from soil by direct culture (× 430).

TABLE I. *Histoplasma capsulatum* Isolated from 2 Specimens by Direct Culture.

Soil No.	Dilution	No. of plates inoculated	No. colonies found	Estimated No. per g of soil
1	1:100	40	30	750
1	1:1000	80	36	4,500
2	1:1000	60	38	6,333

Discussion. It is interesting that the number of colonies of *H. capsulatum* found in the 1:100 dilution of specimen #1 did not show a 10-fold increase over the 1:1000 dilution. This is perhaps the result of a larger amount of other saprophytic fungi present on those plates that were inoculated with a lower dilution of the specimen which either overgrew some of the *H. capsulatum* or produced an inhibitory effect.

Extensive laboratory experience with different soil isolation methods suggests that these successful isolations of *H. capsulatum* were largely due to 3 factors: 1. More rapid germination of *H. capsulatum* when grown on yeast extract medium(6). 2. Relative inhibition of the growth of the saprophytic fungi by the cycloheximide in this medium. 3. Complete inhibition of bacterial growth by the combination of antibiotics used.

Although direct culture methods have not been attempted on other soils, subsequent tests have been done on these 2 soils, where again, *H. capsulatum* was isolated from both soil specimens. It is not known how the efficiency of the direct isolation method will compare with the indirect animal method.

Summary. One hundred and two isolations of *H. capsulatum* were obtained from 2 soil specimens collected from a starling roosting site by direct culture methods. These isolations were obtained on a yeast extract medium containing penicillin, streptomycin, polymyxin B, and cycloheximide. The number of viable particles of *H. capsulatum* was estimated per gram of soil to be 4,500 for soil #1, and 6,300 for soil #2. These numbers

were calculated on the basis of the number of colonies isolated from a 1:1000 dilution of the soil specimens.

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Variations of Acute Iodide Toxicity Among Inbred Strains of Mice. (28965)

FRED G. BOCK AND JOHN J. WRIGHT

Biological Station, Roswell Park Memorial Institute, New York State Department of Health, Springville, N. Y.

Although there is scant information concerning the lethal effects of acute iodide intoxication, most evidence indicates individual variability. In man, individual idiosyncrasies predispose certain persons to iodism, a relatively mild reaction to low levels of administered iodides(1). Occasionally, death occurs in newborn infants whose mothers receive repeated doses of iodides during gestation. The lethal effects in these cases may result from the development of huge goiters which interfere with respiration(2). Such occurrences are infrequent, suggesting that individual sensitivity is involved in these deaths. In rats and dogs, massive doses of iodide cause pulmonary edema, often followed by death; Trotter suggests that production of pulmonary edema by iodide is related to its antithyroid activity(3).

That some aspects of iodine metabolism are under genetic control is indicated by variations of thyroid activity among several lines of inbred mice and their F1 hybrids(4). Evidence presented here shows that acute iodide intoxication is also controlled by genetic factors.

Materials and methods. Mice of various strains (Table I), 30-90 days of age, were injected intraperitoneally with 10% aqueous NaI and observed daily for 14 days. In early experiments, it was noted that all mice killed by the treatment died within 5 days. Thereafter, mice still alive after 14 days were listed as survivors. The LD_{50} and its 99% confidence interval were calculated using the Spearman-Karber method(5). Approximately equal number of males and females were used in each group. With the exception of the ICR Swiss and CFW stocks, the mouse strains had been inbred for at least 20 generations. The CFW stock was obtained from Carworth Farms, inbred for 10 generations and maintained subsequently by random breeding. The ICR Swiss mice have been random bred since the stock was established.

To provide material for genetic analysis, F1 hybrid mice were obtained by reciprocal crosses between the F and CFW stocks. One hundred and seventy-eight of the F1 mice were injected with different levels of NaI to determine the LD_{50}. Other of the F1 hybrids were bred among themselves to provide 170 F2 mice. Finally, the remaining F1 hybrids were mated to animals of the F stock to provide 388 backcross mice. The F2 and backcross mice were injected once with 1.0 g/K of NaI. This dose was found sufficient to kill 100% of the F stock, but none of the CFW mice. The treatment thus served to divide the test animals into 2 groups, those like the F parental stock (sensitive) and those like the CFW stock (resistant).

Three additional groups of CFW mice, 1 month, 4 months, and 8 months of age were treated with either 1.6 or 2.4 g/K of NaI to provide information relating variations in