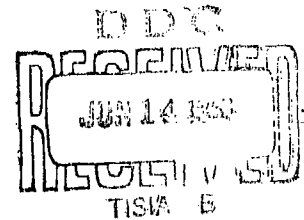


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TECHNICAL MANUSCRIPT 62

GROWTH STUDIES WITH
HISTOPLASMA CAPSULATUM

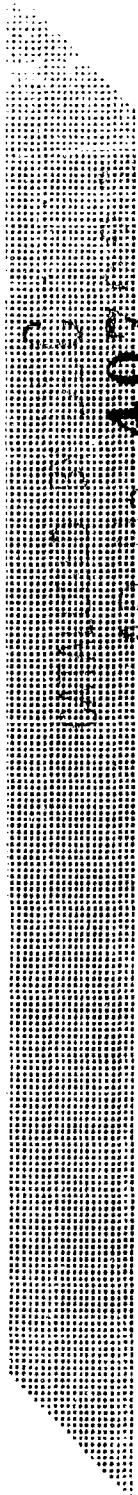
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ABSTRACT

In anticipation of studying the infectivity and virulence of the various forms (yeast cells, microconidia and macroconidia, and mycelial fragments) of Histoplasma capsulatum, the effects of selected medium constituents and cultural conditions on growth and viability of the various forms were determined. Mycelial cultures were fragmented by treatment in a blender to provide small viable units. Hemacytometer counts of such mycelial fragments were compared with viable counts as shown by plating techniques. Although fragmentation increased the number of viable units of mycelial cultures grown in liquid media on the shaker at 27°C, the viable units were only one to five per cent of the hemacytometer counts. Inocula from one-week-old mycelial cultures provided a faster rate of growth in mycelial cultures in various shaken liquid media than did inocula from cultures two to four weeks old. When inocula from surface cultures one, two, three, and four weeks old were used to produce mycelial cultures in peptone yeast extract glucose (PYG) medium on the shaker at 27°C, it was found that (a) maximum viable cell yields occurred earlier with younger inocula, (b) after two weeks, all cultures gave the same maximum viable cell yield, and (c) the culture that showed the earliest maximum cell yield also decreased in viability sooner than other cultures.

The effect of chicken manure extract in liquid media on conversion of yeast cells to mycelia and on viability of mycelial cultures was studied.

GROWTH STUDIES WITH HISTOPLASMA CAPSULATUM

Because of the widely accepted belief that natural infection of man and animals with Histoplasma capsulatum takes place via the respiratory route with microconidia, it seemed desirable to produce mycelial cultures containing large number of these spores for experimental purposes.

During an investigation of the infectivity and respiratory virulence of various mycelial elements of H. capsulatum, it was found that shaken liquid cultures had low viabilities. The viability of these mycelial elements, including macroconidia, microconidia and hyphal fragments, was determined by plate counts after the whole culture had been fragmented in water-cooled blenders. Cultures fragmented for various periods of time up to one hour showed slight increases in hemacytometer counts up to approximately 20 minutes, after which time the counts remained relatively stationary. Viable counts of these fragmented cultures made on blood agar gave very low values—recoveries ranged from 0.5 to 2.0 per cent.

Larsh* found large differences in viabilities of surface cultures of H. capsulatum, depending on the age of surface-grown inoculum. In view of these findings, studies were conducted to determine the effect of age of inocula on the viability of shaken liquid cultures. Mycelia grown on the surface of tryptose agar served as inoculum.

H. capsulatum, strain 3072, was incubated for six weeks at room temperature on a tryptose agar slant in a bottle, and then maintained at 5°C. At one-week intervals, one-fourth of the growth from this stored culture was broken up by shaking with glass beads and buffered saline on a Kahn shaker for three hours. These suspensions were then used to inoculate four groups of six tryptose agar bottle slants.

After incubation of the four groups of bottle slants for one, two, three, and four weeks respectively, all cultures were harvested simultaneously. Growth from individual groups was pooled and broken up by blender action for 20 minutes; suspensions thus prepared were adjusted to the same concentration, based on hemacytometer counts. These suspensions were used to inoculate 100-milliliter liquid cultures containing 2.0 per cent Difco peptone, 0.25 per cent Difco yeast extract, 0.5 per cent dextrose, 50 units penicillin, and 50 micrograms streptomycin per milliliter at a pH of 7.2. The inoculated cultures had an initial count of 3.9×10^8 mycelial particles per milliliter and were incubated at 27°C on a shaker operating at 62 cycles per minute.

* Larsh, H.W. "Natural and experimental epidemiology of histoplasmosis," Annals of the New York Academy of Sciences 89(1) 78-90, 1960.

At weekly intervals for four weeks, one culture from each group was fragmented in a blender, and a hemacytometer count was made. Viable enumerations were made on Rowley's agar containing six per cent sheep blood and on Sabouraud dextrose agar. These plates were incubated at 30°C for seven to nine days before counting. Viable recovery on Sabouraud agar was negligible (Figure 1). All cultures showed significant increases in growth at one week of incubation, and by the end of two weeks of incubation exhibited approximately the same number of viable particles in spite of the fact that the number of viable particles at the time of inoculation ranged from less than 10^2 to 4.4×10^4 per milliliter. In fact, an examination of the data shows that the rate of growth of the various inocula was approximately the same. All cultures showed decreasing numbers of viable particles during the next two weeks. The inoculum for these cultures was composed largely of hyphal fragments, with a few microconidia. A few microconidia and an occasional macroconidium appeared in fragmented shake cultures after two weeks of incubation, and by the third and fourth weeks microconidia comprised an estimated one-fifth of the growth.

In summary, this study indicates that the viability of mycelial inoculum varies with age, and regardless of inoculum size, the same maximum viability is reached after two weeks of incubation in shaken liquid cultures.

Another topic of interest that stems from studies made in Dr. Larsh's laboratory pertains to the effect of chicken manure extract on conversion of the parasitic yeast cells to mycelial forms. The rationale for these studies is based on the fact that the chicken, starling, and bat droppings in soil are frequently associated with epidemic sites of histoplasma infection in man. The chicken manure extract employed in the following study was prepared by centrifuging and autoclaving a 10 per cent suspension of manure obtained from a chicken house on a farm.

A seed culture of Histoplasma capsulatum, strain 18, yeast phase was incubated in Salvin's liquid medium for three days at 35°C on a shaker moving at 60 cycles per minute. This culture served as inoculum for 50-milliliter cultures grown in peptone yeast extract glucose medium, with and without 20 per cent chicken manure extract. After a hemacytometer count on the seed culture was performed, the inoculum was adjusted so that each flask received 10^8 yeast cells per milliliter. In order to convert the yeast cells to mycelium, the flasks were incubated at 27°C on a shaker operating at 62 cycles per minute. At intervals of 4, 8, and 15 days, three cultures of each medium were pooled in blenders and fragmented for 20 minutes. After differential hemacytometer counts of yeast cells and mycelial particles were performed, 10^2 , 10^3 , and 10^4 particles that included yeast cells and mycelial elements were spread on the surface of replicate blood plates. These were then incubated seven to nine days at 30°C before counting.

The hemacytometer counts consisted of hyphal fragments and yeast cells for the four- and eight-day cultures. Microconidia and several mature macroconidia were seen in the 15-day culture containing chicken manure extract; only immature terminal macroconidia were seen in the 15-day culture without the extract.

Figure 2 compares hemacytometer counts of yeast cells and mycelial particles with viable counts in peptone yeast extract glucose medium, with and without chicken manure extract. In medium without chicken manure extract (shown as open points), the hemacytometer counts of yeast cells and total viable counts were similar; however, the hemacytometer counts of mycelial particles remained somewhat lower but approached the yeast and viable counts after 15 days of incubation. In medium containing 20 per cent chicken manure extract (shown as dark points), numbers of yeast cells increased during four days of incubation and then remained relatively stationary. Numbers of mycelial particles increased, however, and after 15 days of incubation were probably the only remaining viable elements. In media containing chicken manure extract, after four days of incubation, yeast inoculum apparently was inhibited and subsequent yeast hemacytometer counts probably represent dead yeast cells. The viable count in this medium after eight days of incubation possibly represents fragments of minute mycelial pellets originally derived from yeast cells converting to mycelial growth. After 15 days of incubation in this medium, the mycelial pellets possibly attained sufficient size to permit a much larger viable count when fragmented.

The most important observation in this study appears to be that in mycelia originating from yeast cells, microconidia and mature macroconidia were found only in medium containing chicken manure extract. These findings indicate a differential inhibition of yeast cells and a favoring of mycelial growth in the presence of the extract, in contrast to a preponderance of yeast growth in cultures without chicken manure extract. The incorporation of avian or bat manure extracts into growth medium may be desirable in future attempts to induce heavy spore production in mycelial cultures.

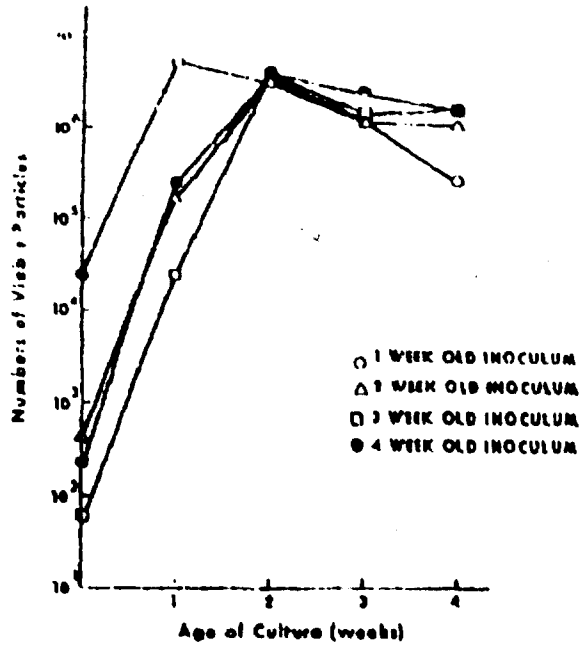


Figure 1. Effect of Age of Mycelial Inoculum on the Viability of *Histoplasma capsulatum* Strain 3072 Grown in Liquid Peptone Yeast Extract Glucose Medium.

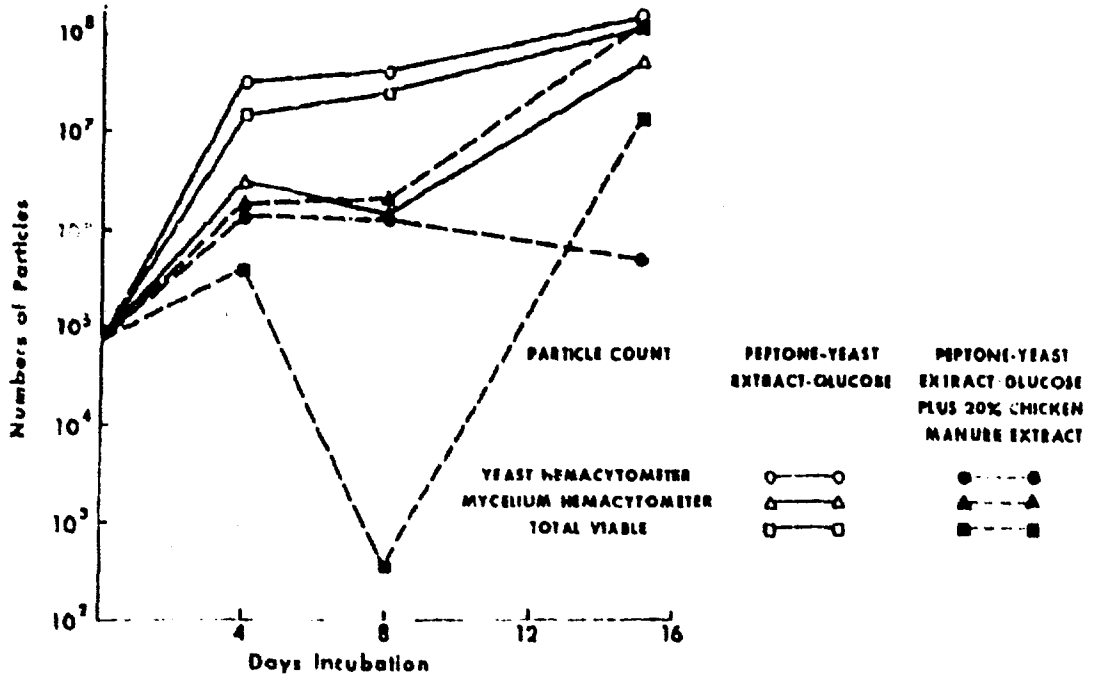


Figure 2. Effect of Chicken Manure Extract on Conversion of *Histoplasma capsulatum* Strain 18 Yeast Cells to Mycelium in Peptone Yeast Extract Glucose Medium.