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# Culture Medium Composition Affects the Lethality of *Cunninghamella bertholletiae* in a Fly Model of Mucormycosis[▽]

Invasive mucormycosis is a mycosis often lethal in immunocompromised patients with hematological malignancies and hematopoietic stem cell transplantation. Among the different genera causing mucormycosis, *Cunninghamella* is associated with the highest overall mortality (4).

Growth studies of filamentous fungi have shown that the composition of culture medium strongly affects growth rates and sporulation (3). It is unclear whether growth in different nutrient media affects the virulence of molds in vivo independently of the in vitro growth rate. To address this question, we used an established model of mucormycosis in *Drosophila melanogaster* (1) to compare the virulence of *Cunninghamella bertholletiae* isolates grown on nutrient-rich medium (yeast extract-agar-glucose [YAG]) with that of the same isolates cultured on nutrient-poor medium (RPMI 1640).

Three clinical *C. bertholletiae* isolates (no. 5, 12, and 17) were studied. *Cunninghamella* sporangiospores were collected from 2-day-old cultures and suspended in 0.85% normal saline. The suspensions were adjusted to a standardized inoculum of $5 \times 10^7$ sporangiospores/ml in 0.85% normal saline for injection into flies (at approximately 800 sporangiospores/fly) and to $10^4$ sporangiospores/ml in yeast nitrogen base for growth rate studies. YAG plates, RPMI 1640 plates without supplemental iron, and RPMI 1640 plates supplemented with 10 μg/ml $FeSO_4 \cdot 7H_2O$ were used for the cultivation of sporangiospores. The growth rates of sporangiospores on poor and rich media at 29°C over 18 h were determined spectrophotometrically. Twenty flies per group were used, and each injection experiment was performed in triplicate on different days. Prism 4 software (GraphPad Software, Inc., La Jolla, CA) was used for the statistical analysis. Statistical significance was defined by a $P$ value of $<0.05$. In the fly infection model, sporangiospores harvested from *C. bertholletiae* cultures grown on RPMI 1640 were more virulent than sporangiospores from the same isolates cultivated on YAG ($P < 0.0001$; median survival time, 3 versus 2 days) (Fig. 1). This effect was not dependent on differences in growth rates (data not shown). Since iron is important for growth and virulence in zygomycosis (5), we speculated that limited iron supply in RPMI 1640 might account for this difference. The addition of 10 μg/ml $FeSO_4$ to the RPMI 1640 plates attenuated the lethality of *Cunninghamella* sporangiospores compared to that of sporangiospores from RPMI 1640 plates without iron, but the lethality was still greater than that of sporangiospores grown in YAG medium ($P = 0.0117$). This phenotype of accelerated lethality was reminiscent of results from previous studies that found that serial passage of *Rhizopus oryzae* on voriconazole-containing agar enhances the lethality of the isolate in fly and mouse models of mucormycosis (2).

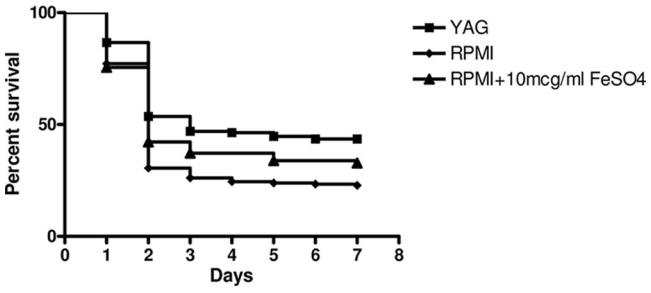


FIG. 1. Sporangiospores from RPMI 1640 and RPMI 1640 supplemented with iron were statistically significantly more lethal to flies than sporangiospores collected from YAG ($P < 0.0001$ and 0.0117, respectively). Data from three experiments per strain were pooled to obtain survival curves indicating the lethality of three *Cunninghamella* strains cultured in poor medium (RPMI 1640), rich medium (YAG), and RPMI 1640 supplemented with iron (RPMI + 10 mcg/ml FeSO4). Data are presented as a Kaplan-Meir plot developed using Prism 4 software (GraphPad Software, Inc., La Jolla, CA), and curves were compared by the log-rank test. Statistical significance was defined by a $P$ value of $<0.05$.

In conclusion, we found that nutritional factors, such as iron availability in growth media, influence the virulence of some molds. Our preliminary data provide a conceptual framework to study the differences in growth media and possibly the contribution of a starvation phenotype to the pathogenesis of molds in experimental systems.

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