Enhanced differentiation of *Fonseca pedrosoi* into sclerotic cells using fetal bovine serum

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Chromoblastomycosis is a chronic fungal disease usually affecting subcutaneous tissues (Fig. 1). *Fonsecaea pedrosoi* is one of the most frequent causative agent of chromoblastomycosis. This fungus is dimorphic and is found inside the host as spherical cells, with a thick and deeply pigmented cell wall known as sclerotic, muriform, fumagoid or Medlar bodies. Attempts to induce the formation of sclerotic bodies in vitro using different approaches have been reported. Acidic medium supplemented with propranolol was described as inductor of sclerotic bodies (1) and replicated using low concentrations of Ca^{+2} (2). Platelet activator factor (PAF), a naturally occurring phospholipid was reported to stimulate the conversion of mycelial to sclerotic forms of *F. pedrosoi* (3). Fetal bovine serum (FBS) has been used as a culture enrichment medium to promote cell differentiation. It is constituted by nutritional factors such as albumin, growth factors, amino acids, sugars, lipids and hormones. In this work we evaluated the effects of FBS to induce sclerotic cells of *F. pedrosoi*.

Figure 1. Chromoblastomycosis in a patient from the Servicio de Dermatología, Hospital Universitario José E. González, Monterrey N.L. México.

Figure 2. (A) Sclerotic cells from tissue on a KOH preparation and (B) Micromorphology of typical conidiophores of *F. pedrosoi*.

Six *F. pedrosoi* isolates obtained from clinical cases in our lab, were grown on Sabouraud-dextrose-agar (SDA) 14 days at 37°C. After the micelial colonies were obtained, we inoculated them into 200 ml of Butterfield and Jong medium (BFJ) (MgSO_4 , 0.1; NH_4NO_3 , 1.5; KH_2PO_4 , 1.8; biotin, 5×10^{-5} ; thiamine-HCl, 10^{-4} ; glycerol, 6.5 supplemented with 800 μM of propranolol and FBS at 2%). FBS-containing cultures were grown at 37 °C; microscopic observation of the cultures was done weekly.

In Fig. 3 we show an example of one of the isolates. Pedunculated spores at the end of hyphae about 5 μm in diameter were observed at third week of incubation. In fig. 3 B, rounded, double-walled spores were seen in the cultures (PAS stain). In fig. 4 the GMS stain evidence the melanized structures. When we shake and separate the articulated to hyphae sclerotic bodies we can see them more rounded. In fig. 5 and 6 the structures rounded, melanized and double walled similar to that observed in human tissues can be observed. The size of these cells was measured and is close to 4-6 μm (Fig. 7).

Figure 3. PAS stain of sclerotic bodies cultured in BFJ medium supplemented with FBS. Structures like aleuroconidia (A, white arrow) and cells with double wall (B, white arrows). 100X

Figure 4. GMS stain for sclerotic bodies cultured in BFJ medium supplemented with FBS . 100 X

Figure 5. Giemsa stain for disarticulated sclerotic bodies cultured in BFJ medium supplemented with FBS. 100 X

Figure 6. Direct microscopic observation of disarticulated sclerotic bodies cultured in BFJ medium supplemented with FBS. 40 X

Figure 7. GMS stain and size determination of sclerotic bodies.

We were able to replicate the experiment of Silva et al. using the BFJ medium. The production of the yeast phase would allow us to test the susceptibility to antimicrobials, already in use and those in experimental phases. We can also use them to study their pathogenic properties and analyze the genes expressed differentially in mycelial and tissue phase looking for new anti-fungal targets for treating this important, but neglected infectious disease.