

Comparing the cytotoxic effect of different lineages of the fungus *Batrachochytrium dendrobatidis*

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The chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) causes chytridiomycosis, which has a negative action in physiological processes in amphibian skin. It is known that *Bd* produces soluble toxic factors that induces apoptosis in lymphocytes and inhibit proliferation of cell lines, which impairs host immune responses. Seven *Bd* lineages were describe worldwide until now, three of those found in Brazil: *Bd*-GPL (Global Pandemic Lineage), *Bd*-Brazil e *Bd*-Hybrid, being the last only found in Brazil. All lineages may differ in virulence. Given the global threat of chytridiomycosis on amphibians, several studies have been performed to understand the variation in virulence of *Bd* lineages. Therefore, we aimed to evaluate the toxicity of *Bd*-Hybrid and *Bd*-GPL lineages. For this, we performed cell viability assays by the MTT (Methyl Thiazolyl Blue) colorimetric method, using the cell line HeLa; *Bd* strains CLFT 024-2 (*Bd*-Hybrid), *Bd*-GPL JEL197 and nonpathogenic chytrid *Homolaphycis polyrhiza* JEL 142 (negative control). The strains were cultivated in plates containing Triptone 1% and incubated at 17°C for ten days. Then, the plates were flooded with 20 mL sterile distilled water during 1 hour. The supernatant harvested from *Bd* cultures was poured in different dilutions on cell culture previously prepared in 96-well plates. After 48 hours, plates were analyzed at 540 nm in spectrophotometer. *Bd*-Hybrid lineage showed more cytotoxic effect than *Bd*-GPL in HeLa cells. One study detected differences in virulence between strains of the same lineage and also differences between different strains. Variation in virulence between the lineages found in Brazil is not well understood. However, the hybrid lineage has shown higher cytotoxic effect, which is a virulence factor. Therefore, we can infer that the hybrid lineage may be more virulent than the GPL lineage. Other studies have detected increased virulence in the strains GPL from another region, while strains of the same lineage show differences in virulence. These results open opportunities to make new analyses about virulence factors lineages from Brazil, which are extremely important for better understanding *Bd* infection dynamics in nature and their effects.

Keywords: Chytridiomycosis; virulence; cytotoxicity; decline.