In Vitro Cultivation of *Balamuthia mandrillaris*: Culture Media, Challenges, and Recent Advances

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Background: *Balamuthia mandrillaris* is a pathogenic free-living amoeba and the causative agent of granulomatous amoebic encephalitis; however, progress in understanding its biology and diagnostics remains limited due to challenges in *in vitro* cultivation. **Objective:** To evaluate the culture media developed for *B. mandrillaris*. **Methods:** This narrative review used MEDLINE, Web of Science, and ScienceDirect. Search terms included "*Balamuthia mandrillaris*", "*in vitro* techniques," "laboratory cultivation," "amoeba culture," and "*in vitro* culture." Articles addressing limitations or advances in cultivation efficiency, reproducibility, and applicability were included. **Results:** Early studies relied on environmental isolation and non-axenic systems, including non-nutrient agar with bacterial feeders and subsequent transfer to mammalian cell cultures. These approaches enabled initial recovery of amoebae but were characterized by slow growth, short survival periods, contamination, and failed axenization [1,2]. Studies using human and animal cell monolayers showed that *B. mandrillaris* replication depends on specific host cells, supported by fibroblasts and endothelial cells but not keratinocytes [3]. Subsequent advances introduced axenic liquid culture systems. Classical media such as BM-3 and modified Chang's supported trophozoite growth but required complex, labor-intensive preparation. A breakthrough was the development of a simplified axenic medium based on Cerva's basal formulation supplemented with Hank's balanced salt solution, which enabled stable, long-term axenic growth across multiple strains [4]. Recent studies used advanced cell-based and tissue-mimicking models, including primary brain tissue models and refined monolayer systems, offering physiologically relevant platforms for cytopathogenicity and drug studies, but remained unsuitable for standardized propagation or routine diagnostics [5]. Despite incremental improvements, *B. mandrillaris* cultivation continues to be limited by slow growth, serum dependence, and incomplete protocol standardization [6-8]. **Conclusions:** The literature demonstrates a gradual transition from poorly reproducible environmental and feeder-dependent systems toward more accessible axenic and advanced host-mimicking media; however, the lack of standardized, defined media remains a critical gap, requiring formulations and harmonized protocols.

Keywords: *Balamuthia mandrillaris*, *in vitro* cultivation, culture media, axenic culture.

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