Afghanistan Journal of Infectious Diseases
AJID
https://ajid.ghalib.edu.af/index.php/ajid

2025 Jan 3(1):10-15

Comparative Analysis of Growth Media for Cultivating *Acanthamoeba*: Implications for Laboratory Diagnosis

Abdolhossein Dalimi*, Ali Reza Mohammady

Department of Parasitology, Faculty of Medical Sciences, University of Tarbiat Modares, Tehran, Iran

ARTICLE INFO

Type: Original Article
Received: 2024/09/17
Accepted: 2024/12/23

*Corresponding Author:
E-mail:
dalimi_a@modares.ac.ir

To cite this article: Dalimi A, Mohammady AR. Comparative Analysis of Growth Media for Cultivating *Acanthamoeba*: Implications for Laboratory Diagnosis.
Afghanistan Journal of Infectious Diseases. 2025 Jan 3(1):10-15.
https://doi.org/10.60141/ajid.76

ABSTRACT

Background: *Acanthamoeba*, a genus of free-living amoebae found in soil, freshwater, and marine habitats, is crucial in environmental studies and medical microbiology due to its potential to cause severe human infections. Cultivating *Acanthamoeba* is crucial for diagnosing infections, guiding treatment, and assessing exposure risks to pathogenic strains. We aimed to compare the different culture media for cultivating *Acanthamoeba* under laboratory conditions.
Methods: We examined ten culture media for *Acanthamoeba* growth, including TSB, Trypticase soy agar (TSA), Trypticase soy yeast (TSY), Trypticase yeast iron-extracted (TYI), Trypticase, yeast maltose extract (TYM), Pepton Yeast Glucose Extract (PYG), Dulbecco's modified Eagle's medium (DMEM), RPMI 1640, Serum Casein Glucose Yeast Extract (SCGY), Coconut powder suspension in NNA medium, as well as non-nutrient agar (NNA) as the primary & control culture.
Results: The results showed varied growth rates of *Acanthamoeba* when cultured in different media, highlighting important trends in their adaptability and growth based on the nutritional composition of the media provided. In TSB, TSA, DMEM/F12 and SCGY media, no growth was observed, suggesting that the combination may lack essential nutrients or conditions necessary for fostering *Acanthamoeba* proliferation. TYI showed an excellent growth rate for *Acanthamoeba*, particularly notable during the first 24-72 hours. The use of maltose as a carbon source and FBS in the NNA environment appears to promote rapid proliferation of *Acanthamoeba*. Coconut powder suspension in NNA medium led to excellent growth rates of *Acanthamoeba*.
Conclusion: The effective cultivation of *Acanthamoeba* in TYI, TYM, and coconut powder highlights the significance of refining nutrient composition to enhance culturing methods.

Keywords: *Acanthamoeba*, Culture media, Parasitology, Coconut powder

Introduction

Acanthamoeba is a genus of free-living amoebae found in soil, freshwater, and marine habitats (1). It is significant in Microbiology and environmental studies for several reasons. As a free-living amoeba, it is widely distributed in various environments, including soil and water. It is an opportunistic pathogen known to cause serious human infections such as keratitis and granulomatous amebic encephalitis (GAE), which can be life-threatening. Its ability to survive in harsh conditions makes it a model organism for studying protozoan biology and ecology, as well as the interactions between pathogens and hosts (1).

To culture and sustain *Acanthamoeba* in the lab, various media are used. These include non-nutrient agar (NNA), PYG Medium, Neff Medium, Cysts Medium (CM), and

Cyst Enumeration Medium. NNA medium, is a basic culture medium used to culture *Acanthamoeba*. It is a simple medium that allows the amoeba to grow while inhibiting the growth of other organisms, like bacteria. NNA is often supplemented with bacteria like *Escherichia coli* to provide additional nutrients. PYG Medium is beneficial for promoting growth and observing morphology, while Neff Medium is commonly employed for general *Acanthamoeba* cultivation. CM is designed to stimulate and observe cyst formation, and Cyst Enumeration Medium creates the ideal conditions for cysts to germinate and develop into trophozoites (2).

Acanthamoeba cultivation is important for scientific research, medical diagnosis, environmental research and public health. Cultures derived from clinical specimens, such as corneal scrapings, are essential for diagnosing infections and guiding treatment options (3). Cultures of environmental samples help to assess the risks associated with exposure to pathogenic strains and help in shaping public health plans (4). In addition, culture of the parasite to investigate the biology, life cycle and pathogenic strategies of *Acanthamoeba* can enhance therapeutic approaches and preventive strategies. Furthermore, understanding its ecological role, including its interaction with bacteria and other microorganisms, is key to assessing freshwater and marine ecosystems (5). Moreover, the culture of *Acanthamoeba* serves as a model organism to study the factors influencing the survival of amoeba and the development of new antimicrobial treatments, which may potentially lead to the discovery of effective drugs for *Acanthamoeba* infections and other amoebic diseases (5).

In recent years, some researches have presented some studies on the growth of the *Acanthamoeba* parasite in vitro. Niyyati et al. explored methods for cultivating *Acanthamoeba* strains without other microorganisms (axenic cultivation) and tested their pathogenicity (6). Eroğlu et al. examined various growth media, both axenic and monoxenic, for cultivating *Acanthamoeba* (7). Peterz et al. compared fluorescence microscopy techniques with different culture methods for diagnosing *Acanthamoeba* (8). Besides, Ziaei Hezarjaribi et al. conducted research to evaluate how various culture media affect the growth and reproduction of the T4 genotype of *Acanthamoeba* (9).

There has been limited research on comparing various media that can be used for this purpose. This highlights the importance of further investigation into this issue. We aimed to evaluate the different culture media for *Acanthamoeba* cultivation in laboratory environments.

Materials and Methods

Parasite

Acanthamoeba spp. used in the present study was prepared from the Parasitology Laboratory of the Faculty of Medical Sciences of Tarbiat Modares University, Tehran, Iran, isolated from the environment sample and confirmed by sequencing as T4 genotype (10). The parasite was being passaged in laboratory in NNA medium with heat killed *E. coli* and incubated at 26 °C for up to 3 days.

Culture media preparation

In the present study, ten culture media, including Trypticase broth (TSB), Trypticase soy agar (TSA), Trypticase soy yeast (TSY), Trypticase yeast iron-extracted (TYI), Trypticase yeast maltose extract (TYM), Pepton Yeast Glucose Extract (PYG), Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 (Roswell Park Memorial Institute), Serum Casein Glucose Yeast Extract (SCGY), Coconut powder suspension in NNA medium, as well as non-nutrient agar (NNA) as the primary and control culture, were examined.

TSB, TSA, TSY, TYI, TYM, PYG, DMEM, SCGY were prepared according to the reported instructions (6, 11-14). TYM

medium (Remel, Lenexa, KS, USA) was purchased as ready to use. RPMI 1640 medium (Gibco, Eggenstein, Germany) was supplemented with 10% fetal bovine serum, 100 µg/mL streptomycin, and 100 IU/mL penicillin. All culture media were stored in an incubator where the pH level ranged from 6.9 to 7.2 and the temperature was set at 26 °C. The samples were incubated for at least 24 hours and parasite growth was monitored every day.

Assessment of the parasite growth

The parasite was first propagated in NNA medium, and ten liquid culture media were assessed for its propagation. NNA medium was selected as control group. The experiment was conducted in a 96-well microplate with three replicates per medium, incubated at 26 to 30 °C for 72 hours. Each medium received 200 µl along with 2 x 10^5 cysts of parasite in three wells. After 24, 48, and 72 hours, the growth of the parasite was evaluated by placing 20 µl from each well on a Neubauer chamber slide and comparing it with the NNA culture medium after counting.

Results

The results indicated varied growth rates of *Acanthamoeba* when cultured in different media, highlighting important trends in their adaptability and growth based on the nutritional composition of the media provided (Table 1).

Table 1: *Acanthamoeba* growth rate in different culture media.

Culture media	***Acanthamoeba growth rate***
Trypticase broth (TSB)/ or in combination with blood	No growth
Trypticase soy agar (TSA) mixed with blood	No growth
Trypticase soy yeast (TSY)	Low growth
Trypticase yeast iron-extracted (TYI)/ with FBS/ in NNA medium	Excellent growth (in NNA the first 24-72 hours is good)
Trypticase, yeast maltose extract (TYM) pure/with FBS/in NNA medium	Excellent in the first 48-72 hours
Pepton Yeast Glucose Extract (PYG)	Moderate (late) growth
DMEM/F12	No growth
RPMI 1640	Moderate growth
Serum-casein Glucose Yeast Extract (SCGY)	No growth
Coconut powder suspension in NNA medium	Excellent growth

In TSB no growth of *Acanthamoeba* was observed, indicating that this combination may lack essential nutrients or conditions necessary for development *Acanthamoeba* proliferation. TSA was similar to the TSB condition, *Acanthamoeba* growth was absent. TSY medium exhibited a low *Acanthamoeba* growth rate, implying that while some essential nutrients are present, they might not be sufficient or optimal for robust growth.

In TYI an excellent growth rate for *Acanthamoeba* was achieved, particularly notable during the first 24-72 hours. This indicates that the combination of trypticase, iron, and FBS provides a highly favorable condition for *Acanthamoeba* growth. TYM with FBS, like TYI, TYM also demonstrated an excellent *Acanthamoeba* growth rate in the initial 48-72 hours. The use of maltose as a carbon source and FBS in the NNA environment promotes rapid proliferation of *Acanthamoeba*.

The growth rate of *Acanthamoeba* in PYG medium was classified as "moderate" and occurred late in the growth period, suggesting that PYG is not a fast-acting medium and may be less effective than

others in the short term, requiring more time for the parasite to grow. DMEM medium showed no growth of *Acanthamoeba*, indicating that the specific nutrient formulation is not suitable for the proliferation of this amoeba. Similar to DMEM/F12, SCGY resulted in no growth of *Acanthamoeba*, suggesting that the combination of serum casein and glucose is insufficient for supporting *Acanthamoeba* growth. The results suggest a moderate growth rate for *Acanthamoeba* in RPMI Medium. Remarkably, coconut powder suspension in NNA medium led to excellent growth rates of *Acanthamoeba*. The presence of coconut powder might provide unique growth factors or nutrients that are highly beneficial to *Acanthamoeba* proliferation.

Discussion

The findings from the various culturing media provide valuable insights into the nutritional needs and optimal growth conditions for *Acanthamoeba* proliferation. Analyzing each medium reveals factors that either promote or hinder growth. The standard nutrient media used for *Acanthamoeba* generally includes peptone, yeast extract, and glucose, typically at higher concentrations than those found in bacterized cultures (15).

Our results indicate that *Acanthamoeba* did not grow in TSB medium, implying that it might not meet its metabolic requirements. Despite TSB being nutrient-rich, adding blood does not appear to provide beneficial growth factors, possibly due to unfavorable chemical composition or inadequate nutrient bioavailability.

Similarly, TSA also failed to support *Acanthamoeba* growth, reinforcing the idea that the solid form may limit nutrient availability or that the overall formulation is inadequate for this organism. The lack of growth in both liquid and solid media highlights the urgent need to reevaluate nutrient formulations for effective *Acanthamoeba* culturing.

The low growth rate observed with TSY indicates that while it might provide some essential nutrients, the quantities are insufficient for vigorous growth. This suggests that specific growth factors or additional nutrients may be lacking, hindering *Acanthamoeba*'s ability to thrive. In contrast, the excellent growth rate in TYI medium indicates highly favorable conditions for *Acanthamoeba*. The combination of TYI and fetal bovine serum (FBS) appears to supply the critical nutrients and growth factors necessary for rapid proliferation, especially during the initial 24-72 hours. This implies that iron is vital for supporting metabolic processes and may enhance *Acanthamoeba* viability.

The strong growth rate in TYM mirrors the success of TYI, suggesting that the maltose extract also provides essential carbohydrates and nutrients that promote well *Acanthamoeba* growth. The consistent performance of both formulations indicates that *Acanthamoeba* species can effectively utilize different carbon sources. A study reported TYM as the best environment for short-term parasite proliferation (9).

The results indicate the average growth rate of *Acanthamoeba* in RPMI culture medium. However, additional details may be necessary to fully describe the observed growth levels. The designation of an "average growth rate" for RPMI 1640 suggests it provides a baseline growth capability but lacks the robust proliferation found in more effective media. This medium is particularly suitable for growing *Acanthamoeba* when large quantities are needed quickly, making RPMI 1640 a solid choice (16, 17).

PYG medium is highly recommended for *Acanthamoeba* cultivation. However, this study revealed only moderate growth in later stages, suggesting it supports some *Acanthamoeba* growth but is not ideal for short-term proliferation. The delayed growth may indicate slower metabolic activity due to specific carbohydrates or amino acids present (7, 8).

The absence of *Acanthamoeba* growth in

DMEM/F12 Medium, which is generally diverse, indicates that its specific formulation may not meet the needs of *Acanthamoeba*. While commonly used in cell culture, it might lack certain nutrients or essential factors necessary for amoebic growth.

The lack of growth in SCGY medium indicates a likely deficiency in critical nutrients or an imbalance in the combination of casein and glucose that does not support *Acanthamoeba* activity.

Notably, the impressive growth rate observed in coconut powder suspension in NNA medium suggests that coconut powder may offer unique nutritional benefits, potentially through bioactive compounds or a rich array of carbohydrates and fatty acids that significantly support *Acanthamoeba* growth.

Conclusion

Acanthamoeba has specific nutritional needs that can vary considerably based on the composition of the culturing media. The successful growth of *Acanthamoeba* in TYI, TYM, and coconut powder emphasizes the importance of optimizing nutrient composition for effective culturing techniques. More investigations are required to pinpoint the exact factors that lead to improved growth of certain media used in experiments. It is important to carry out quantitative research, particularly in areas where the parasite has proliferated significantly. Understanding this could impact both research and clinical practices involving *Acanthamoeba*.

Acknowledgment

This article is part of a PhD thesis in Medical Parasitology financially supported by Tarbiat Modares University (Tehran, Iran). The authors wish to thank the staff of the Parasitology Department of the Medical Sciences Faculty for their kind assistance.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Lorenzo-Morales J, Khan NA, Walochnik J. An update on *Acanthamoeba* keratitis: diagnosis, pathogenesis and treatment. Parasite. 2015; 22: 10.
2. Siddiqui R, Khan, NA. Biology and Pathogenesis of *Acanthamoeba*. Clin Microbiol Rev. 2012; 25(1), 132-165.
3. Fanselow N, Sirajuddin N, Yin XT, Huang AJW, Stuart PM. *Acanthamoeba* keratitis, pathology, diagnosis and treatment. Pathogens. 2021 Mar; 10(3): 323.
4. Niyyati M, Hossain MB. *Acanthamoeba*: A free-living amoeba and its environmental role. Environ Monitor Assess. 2018; 190(6), 340.
5. Khan NA. *Acanthamoeba* infections in humans: A potential threat. Microbiol Today. 2006; 33(2), 68-71.
6. Niyyati M, Abedkhojasteh H, Salehi M, Farnia S, Rezaeian M. Axenic cultivation and pathogenic assays of *Acanthamoeba* strains using physical parameters. Iran J Parasitol. 2013; 8(2):186-9.
7. Eroğlu F, Evyapan G, Koltaş İS. The Cultivation of *Acanthamoeba* Using Different Axenic and Monoxenic Media. Mid Blac Sea J Health Sci. 2015; 1(3): 13-17.
8. Peretz A, Geffen Y, Socea SD, Pastukh N, Graffi S. Comparison of fluorescence microscopy and different growth media culture methods for *Acanthamoeba* keratitis diagnosis. Am J Trop Med Hyg. 2015; 93: 316-318.
9. Ziaei Hezarjaribi H, Dadimoghaddam Y, Hassanjani Saravi K, Faridnia R, Kalani H, Fakhar M, Jorjani O, Saberi R, Tolouei E, Bazgir M. The effectiveness of different culture media on the growth and proliferation of *Acanthamoeba* T4 genotype. Comp Clin Path. 2021; 30:515–518.
10. Mohammady A, Dalimi A, Ghafarifar F, Akbari M, Pirestani M. Genotyping of *Acanthamoeba* isolated from hospital environments and thermal water of

recreational baths in Markazi Province, Iran. Iran J Parasitol. 2023; 18 (1):38–47.
11. Penland RL, Wilhelmus KR. Comparison of axenic and monoxenic media for isolation of *Acanthamoeba*. J Clin Microbiol. 1997;35(4):915-22.
12. Garcia LS. Parasite Recovery: Culture Methods, Animal Inoculation, and Xenodiagnosis. Diag Med Parasitol. 2016. p. 156-82.
13. Diamond LS, Harlow DR, Cunnick CC. A new medium for the axenic cultivation of Entamoeba histolytica and other Entamoeba. Trans R Soc Trop Med Hyg . 1978;72(4):431-2.
14. Clark CG, Diamond LS. Methods for cultivation of luminal parasitic protists of clinical importance. Clin Microbiol Rev. 2002;15(3):329-41.
15. Schuster FL. Cultivation of pathogenic and opportunistic free-living amebas. Clin Microbiol Rev. 2002; 15: 342-354.
16. Limoncu ME, Ozbilgin A, Balcioglu IC, Ozbel Y. Evaluation of three new culture media for the cultivation and isolation of Leishmania parasites. J Basic Microbiol. 2004; 44: 197-202.
17. Sharief AH, Khalil EA, Omer SA, Abdalla HS. Innovative serum-free medium for in vitro cultivation of promastigote forms of Leishmania species. Parasitol Int. 2008; 57; 138-142.