Original Article

Enrichment of *Acanthamoeba* Culture Medium Using TYIS 33 Medium: a Step toward a Successful Axenic Cultivation

Maryam Niyyati[1*], Samira Dodangeh[1]

[1] Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Background: Acanthamoeba-related disease have a poor prognosis according to many previous studies. Thus researches regarding biochemical and molecular aspects of this organism are a high priority. To this end achieving high amount of amoebae in culture is the first step for such studies. The main aim of the present research was to address the usage of TYI-S-33 (Tripticase, Yeast extract, iron-serum) medium as an enrichment component for achieving high and fast growth of trophozoites in agar culture medium within 24 hours.

Materials and Methods: Overall, 10 *Acanthamoeba* strains were cultured, cloned and genotyped and the cultures were then enriched with addition of TYI-S-33 medium. Amoebae growth was then monitored daily. Ten plates also were used without addition of TYI-S-33 medium.

Results: The result of the present research revealed that addition of TYI-S-33 medium is a promising approach for obtaining 100% trophozoites within 24 hours of culture.

Conclusion: To the best of our knowledge this is the first report of successful achieving high amount of trophozoites within short time that able researchers to arrange molecular and biochemical assays.

Keywords: *Acanthamoeba*, TYI-S-33 medium, Culture, Genotypes, Trophozoites

***Corresponding Author:** Maryam Niyyati, Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: Maryamniyati@yahoo.com

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Introduction

Acanthamoeba spp. is a ubiquitous protozoan eukaryotic parasite with a potential of severe human diseases including amoebic keratitis and granulomatous encephalitis[1-3]. Unfortunately, all *Acanthamoeba* related diseases have poor prognosis and many patients show no improvement even after combination therapy[4]. It should be mention that during recent years scientists have recommended for searching new diagnostic tools and therapy approaches, however this could be done by a successful culture[5].

Classification of *Acanthamoeba* is mainly based on sequencing of a diagnostic fragment 3 of 18S rRNA gene and 18 genotypes have been identified so far[1-6]. It should be mention that *Acanthamoeba* isolation from both environmental and clinical samples needs cultivation of samples into non nutrient agar[6,7]. This medium consist of low nutrient molecules mainly due to avoid bacteria and fungi proliferation[8]. On the other hand, amoebae needs some sources of food and thus adding gram negative bacteria such as *Escherichia coli* could help the amoebae to growth in the medium[6-8]. However some researches especially in the molecular and biochemical fields need a high amount of amoebae in culture medium[2].

In this regards several studies showed that many

Acanthamoeba strains are resistance to growth appropriately in liquid medium such as protes pepton, yeast extract and glucose (PYG medium). Accordingly a research conducted by Rezaeian *et al.* Showed that *Acanthamoeba* could not adapt to liquid medium readily[1-3].

TYI-S-33 (Tripticase, Yeast extract, iron-serum) contains rich substances such as yeas extract and tripticase which could support the growth of *Acanthamoeba* spp. It should be mention that researchers were mainly uses PYG for axenic cultivation of the amoeba however failure in this culture medium is a usual phenomenon.

Overall the present research aimed to investigate the efficacy of addition of TYI-S-33 medium in non-nutrient agar. To the best of our knowledge the present research is the first to introduce achieving a high amount of trophozoites within 24 hour of cultivation using TYI-S-33.

Methods

In this experimental study TYI-S-33 (Tripticase, Yeast extract, iron-serum 33) were prepared according to previous studies [10]. Briefly, TYI-S-33 medium were performed using distilled water (100 ml), 0.1 gram (g) of potassium phosphate, dibasic; 0.06 g of potassium phosphate, monobasic; 0.2 g of sodium chloride; 0.2 g of casein digest peptone; 2 g of yeast extract; 1 g of glucose; 0.1 g of L-cysteine[10]. *Acanthamoeba* type strains have been isolated from clinical and environmental sources in our previous studies. Briefly, three *Acanthamoeba* were isolated from corneal samples of amoebic keratitis patients and 7 *Acanthamoeba* were isolated from different kind of water sources in Tehran, Iran. *Acanthamoeba* strains were cloned and they were characterized at the genotype level based on sequencing of 18S rRNA gene and homology analysis in BLAST program. It should be mention that all of isolated *Acanthamoeba* plates were free of bacteria and fungi and they were kept in 1% non-nutrient agar (Bacto-agar, Difco). The medium was prepared using distilled water and bacto-agar according to previous studies. This medium allows *Acanthamoeba* spp. to grow, but as it is non – nutritious medium they limit the growth of bacteria and fungi.

All of the isolates failed to grew on PYG medium and thus we have added 5 µl and 10 µl of TYI-S-33 medium in the corner of cloned plates and they were monitored from the next day till several weeks. The control plates contained only bacteria without addition of TYI-S-33 medium.

Results

All of isolates have been cloned within 5 weeks. Sequence analysis revealed that genotypes were belonged to T3, T4 and T5 types. Addition of TYI-S-33 liquid culture to the bactoagar-agar medium lead to high growth of amoebae within one day after enrichment. This finding could lead to achieving high amount of trophozoites compatible with amoebae growth in PYG medium (Figure 1) (Table 1) and thus this could be an alternative approach for achieving trophozoites for biochemical or molecular analysis.

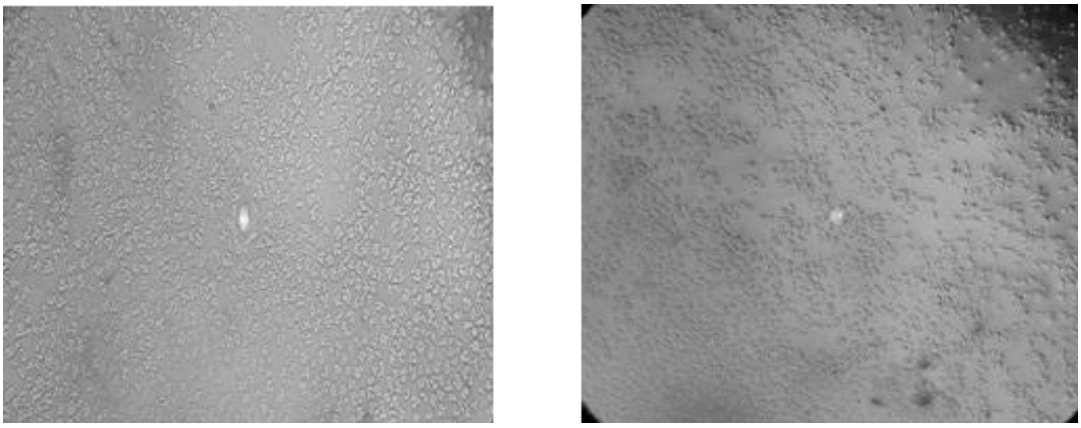

Figure 1. *Acanthamoeba* trophozoites in bacto-agar medium with addition of TYI-S-33 medium after 24 hours.

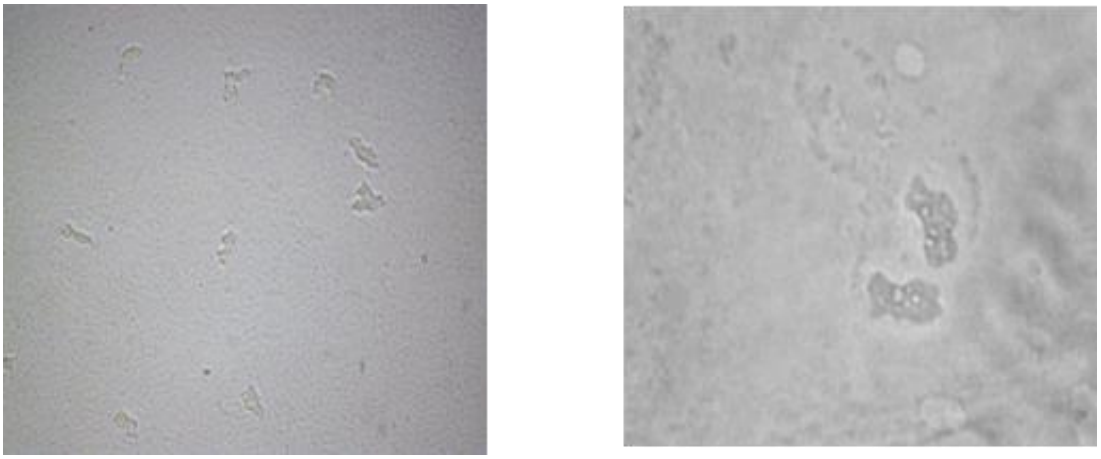

Figure 2. ***Acanthamoeba*** **trophozoites in agar medium without addition of TYI-S-33 medium after 24.**

Table 1: *Acanthamoeba* genotypes and their original sources.

Acanthamoeba **code**	**Sources**	**Genotypes**
KS1	Keratitis patient	T4
KS43	Keratitis patient	T4
KS15	Keratitis patient	T4
WS3	Park water	T5
WS8	Pond water	T4
WS19	Park water	T4
WS60	Swimming pool	T3
W76	Swimming pool	T4
WS77	Lake water	T5
WS 90	River	T3

The growth measurement is qualitative phenomenon and the analysis is based on comparison with control plates[1]. It should be mention addition of food sources such as *Escherichia coli* or other gram negative bacteria can lead to little growth of amoebae in culture medium and may take long time (Figure 2). Both concentrations (5 and 10) were showed the same result.

Discussion

Acanthamoeba app. is a leading cause of amoebic keratitis in Iran and worldwide. Other diseases related to the mentioned amoebae is amoebic granulomatose encephalitis and skin ulcers[1,5,9-12]. Todate, molecular and biochemical analysis of such amoebae is a research priority[6,7,13]. Additionally, pathogenicity assays and effect of endosybionts of *Acanthamoeba* is still vague and needs more researches. Relationship of amoebae with many other microorganisms such as bacteria, viruses and yeasts needs a high amount of amoebae to be involved in analysis[1].

The first step in biochemical and molecular analysis is to achieve high amount of amoebae in culture medium. However, axenic cultivation of *Acanthamoeba* spp. in PYG medium is a challenging process[13]. Indeed, axenic cultivation depends on amoeba strains and also adaptation behavior of the amoebae in culture. According to studies in this regard many strains of *Acanthamoeba* fail to adopt to liquid medium such as PYG and this may take time as long as six months or more[14].

In a previous studies in Iran it has shown that only 1 from 10 *Acanthamoeba* type strain adopted to axenic cultivation in PYG medium after six month[14,15]. This is important since making several cultures could attenuate amoebae pathogenicity and thus fast achievement of amoebae could lead to precise mechanisms of amoebae in cell culture or in *in vivo* models.

According to Khan et al. studies resistance of many

Acanthamoeba strain to grow on PYG is problematic issue and thus finding a new solution is of utmost importance [3,5,6]. The present study reflect for the first time that addition of rich mediums such as TYI-S-33 could be an alternative approach for achieving trophozoites in agar in 24 hours. It should be mention that other medium such as RPMI should be test for this purpose in future.

Conclusion

Overall, the present research highlights the advantages of using TYI-S-33 medium for enrichment of plate cultures and also achieving trophozoites in short time.

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