Research article

Growth comparison of Acanthamoeba genotypes T3 and T4 in several culture media

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ABSTRACT

Acanthamoeba causes severe diseases such as Granulomatous Amebic Encephalitis (GAE) and Acanthamoeba keratitis (AK). Improving the culture media classically used for this amoeba could help to identify it quickly and facilitate its study as a biological model. The purpose of this study was to compare the growth of two Acanthamoeba genotypes (T3 and T4) in several culture media. Acanthamoeba griffini (T3 genotype) and Acanthamoeba castellanii (T4 genotype) were cultured in PYG, TSY, TYI-S-33, RPMI, and RPMI-FBS medium. The number of amoebas grown in different culture media was counted and compared to each other for 14 days. Findings in this research revealed the highest growth in RPMI-FBS medium. For this reason, we can recommend this culture medium to promote the growth of Acanthamoeba in its biological studies.

1. Introduction

Acanthamoeba is one of the most common opportunistic free-living amoebae with ubiquitous presence in various environmental sources such as air, soil, and water. Acanthamoeba causes severe diseases such as Granulomatous Amebic Encephalitis (GAE), cutaneous lesions, and Acanthamoeba keratitis (AK) that is associated with contact lens use or corneal trauma (Marciano Cabral and Cabral, 2003). This amoeba is also used for biological studies, and there are many studies about the interaction of Acanthamoeba with other microorganisms (Siddiqui and Khan, 2012; Axelsson-Olsson et al., 2005; Abrahao; et al., 2014). Currently, there is no fully efficient treatment scheme to treat Acanthamebiasis, more research is needed to understand its molecular and biochemical processes and thus efficiently control and treat infections. It is necessary to improve the culture media to grow faster and therefore to carry out biochemical and molecular studies in less time (Baig, 2018; Khan, 2003; De Jonckheere, 1980). The non-nutrient agar (NNA) and Peptone-yeast-glucose (PYG) media have been introduced as standard culture media for Acanthamoeba (Garcia, 2007; Axelsson-Olsson et al., 2009; Sheng et al. 2009; Panjiwani, 2010). In this study, Acanthamoeba griffini (T3 genotype) and Acanthamoeba castellanii (T4 genotype) were cultured in PYG, Trypticase Soy Broth with Yeast Extract (TSY), Trypticase-Yeast Extract-Iron-Serum (TYI-S-33), Roswell Park Memorial Institute Media (RPMI) and RPMI-FBS medium. Then the number of amoebas in different culture media was counted and compared to each other for 14 days. TYI-S-33 culture media is used for the axenic culture of Entamoeba histolytica (Diamond, 1982). It should be noted that a modified form of this culture medium is also used for the cultivation of Giardia and Trichomonas parasites (Clark and Diamond, 2002). TSY, in addition to being used for the growth of Acanthamoeba, is widely used to study symbiotic interactions between this organism and bacteria (Lagkouvardos et al. 2014). RPMI 1640, also known as RPMI medium, is a growth medium used in cell culture. It can also be used to culture leishmania and malaria parasites. RPMI-FBS provides a very rich medium for the growth of parasites and cells. This culture medium is more accessible compared to others and is commercially available (Schuster, 2002; Lemesre et al., 1988). Additionally, the growth of both Acanthamoeba genotypes on non-nutrient agar (NNA) was evaluated by replacing the use of the bacterial lawn with a certain amount of each described culture medium and the growth was quantified on specific days.

2. Material and methods

2.1. Preparation of Acanthamoeba for cultivation

Both Acanthamoeba griffini (genotype T3) (MH938695) and Acanthamoeba castellanii (genotype T4) (MH938701) were used during the experiments. They were preserved and grown in nutrient-free agar (NNA) in the facilities of the Parasitology Laboratory, University of Tehran. They were harvested from the plate surface using Cell Scrapers and then,

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washed by centrifugation three times with amoeba saline (ATCC 1323 Page's amoeba saline) and counted in the Neubauer chamber.

2.2. Preparation of culture media

Culture media were prepared according to protocols (PYG) (ATCC 712), (TSY) (ATCC 993), (TYI-S-33) (Diamond, 1982). The RPMI 1640 medium and Fetal Bovine Serum was purchased from Sigma-Aldrich. One ml of prepared culture media were added to each of three wells of a 24 well plate (SPL Cell Culture Plate). Finally, 0.1 mg/mL penicillin (Sigma-Aldrich), 0.1 mg/mL streptomycin (Sigma-Aldrich), were added to all wells. The amount of FBS added to the RPMI medium was twelve percent. Finally, \(5 \times 10^4\) of each amoeba in 100 μl and added to each well. Plates were incubated at 28°C for 2–14 days.

2.3. Qualitative study of amoeba growth in non-nutrient agar (NNA) culture media

Fifty μl of each culture medium (PYG, TSY, TYI-S-33, RPMI and RPMI-FBS) and \(5 \times 10^4\) trophozoites were added in five plates with NNA medium. later the growth was evaluated using an inverted microscope (AE31 Elite Binocular) every day for 14 days. As a control, NNA standard medium supplemented to an Escherichia coli lawn was used.

3. Results

3.1. Effect of culture media on amoeba growth

After counting the number of amoebae recovered every 24 h for 14 days, the results based on the collected data are shown in Tables 1 and 2. The mean for Acanthamoeba castellanii (genotype T4) was 8.77, and for Acanthamoeba griffini (genotype T3) it was 8.25. Based on the independent t-test, the p-value was 0.612 > 0.05 with \(T = 0.510\). This result indicates that there was no significant difference between the two groups (Table 1).

Based on one-way analysis of variance (One-way ANOVA) to study the difference in the number of amoeba on different days and according to the amount of \(F = 25.008\) and p-value 0.000 < 0.05, there is a significant difference in amoebic growth on different days. This amount was higher on the sixth day (Table 2).

3.2. Effect of culture media on amoeba growth in NNA culture media

In the study where amoebas were inoculated into added NNA plates from each culture medium, the highest growth was observed on the plate containing RPMI-FBS, PYG, TSY, TYI-S-33, and RPMI Media. This result

| Table 1. T-test for equality of means. |
|---------------------------------------|
| T  | DF  | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | N  | Mean  | Std. Deviation | Name of amoeba |
|---------|------|----------------|-----------------|-----------------------|------------------------------------------|-----|-------|----------------|----------------|
| 0.510   | 78   | 0.612          | 0.52500         | 1.02984               | 1.52524, 2.57524                        | 40  | 8.775 | 4.92241       | Acanthamoeba castellanii (T4 genotype) |
| 40     | 8.250 | 4.26524       |                 |                       |                                         |     |       |               | Acanthamoeba griffini (T3 genotype) |

| Table 2. One-way analysis of variance (One-way ANOVA) to study the growth of Acanthamoeba trophozoite at different time points. |
|-----------------------------------------------------------------------------------------------------------------------------------|
| Day | N | Mean | Std. Deviation | 95% Confidence Interval for Mean | Minimum | Maximum | F    | SIG |
|-----|---|------|----------------|---------------------------------|--------|--------|------|-----|
|     |   |      |                | Lower Bound                        |        |        |      |     |
|     |   |      |                | Upper Bound                        |        |        |      |     |
| 1.00 | 10 | 5.0000 | .00000 | 5.0000                        | 5.0000 | 5.0000 | 25.008 | 0.000 |
| 2.00 | 10 | 7.7000 | 1.88856 | 6.3490                        | 9.0510 | 5.00    | 11.00 |     |
| 4.00 | 10 | 12.2000 | 3.55278 | 9.6585                        | 14.7415 | 8.00    | 19.00 |     |
| 6.00 | 10 | 13.6000 | 3.97772 | 10.7545                        | 16.4455 | 8.00    | 21.00 |     |
| 8.00 | 10 | 12.7000 | 3.16403 | 10.4366                        | 14.9634 | 9.00    | 17.00 |     |
| 10.00 | 10 | 9.1000 | 2.42441 | 7.3657                        | 10.8343 | 6.00    | 13.00 |     |
| 12.00 | 10 | 5.5000 | 1.71594 | 4.2725                        | 6.7275 | 3.00    | 8.00  |     |
| 14.00 | 10 | 2.3000 | 1.56702 | 1.1790                        | 3.4210 | 1.00    | 5.00  |     |
| Total | 80  | 8.5125 | 4.58394 | 7.4924                        | 9.5326 | 1.00    | 21.00 |     |

| Table 3. One-way analysis of variance (One-way ANOVA) to study the differences in numbers of Acanthamoeba trophozoite at (1–14) days of culturing in different media. |
|---------------------------------------------------------------|
| culture media | N  | Mean   | Std. Deviation | 95% Confidence Interval for Mean | Minimum | Maximum | F    | SIG |
|----------------|-----|--------|----------------|---------------------------------|--------|--------|------|-----|
|                |     |        |                | Lower Bound                      |        |        |      |     |
|                |     |        |                | Upper Bound                      |        |        |      |     |
| PYG            | 16  | 10.0625 | 4.37369 | 7.7319                        | 12.3931 | 4.00    | 17.00 | 2.879 | 0.028 |
| TSY            | 16  | 9.3125 | 4.04506 | 7.1570                        | 11.4680 | 3.00    | 15.00 |     |
| TYI-S-33       | 16  | 7.8750 | 4.12916 | 5.6747                        | 10.0753 | 1.00    | 14.00 |     |
| RPMI           | 16  | 5.8750 | 2.65518 | 4.4602                        | 7.2898 | 1.00    | 9.00  |     |
| RPMI + FCS     | 16  | 10.5625 | 6.33476 | 7.1869                        | 13.9381 | 1.00    | 21.00 |     |
| Total          | 80  | 8.8250 | 4.61910 | 7.7971                        | 9.8529 | 1.00    | 21.00 |     |
was consistent with the results of amoeba cultivation in the axenic culture media. There was no difference in growth between the *Acanthamoeba griffini* (T3 genotype) and *Acanthamoeba castellanii* (T4 genotype).

4. Discussion

*Acanthamoeba* is one of the free-living amoebas that cause severe diseases and used for biological studies. Currently, more research is needed to control this opportunistic amoeba, in addition to culture media diseases and used for biological studies. Currently, more research is interpreted the data; Wrote the paper.

Increase the amoeba growth. On the other hand, growth in NNA medium and/or cysts found in corneal scraping samples. In the study of Axelsson-Olsson et al. (2009), they proposed a novel and simple technique useful in the diagnosis of AK, promoting the growth of trophozoites results of Ero et al., 2009). The results of the present study were consistent with the other culture media. Also, there were no significant differences in growth in PYG culture medium as compared to our study (Ergölu et al., 2015). In another study by Peretz et al., it was observed that culture in PYG and TYI-S-33 medium could help to diagnose *Acanthamoeba* keratitis alongside the calcfluor white staining and fluorescence microscopy. PYG culture media was reliable, cost-effective, efficient, and straightforward method for the detection and diagnosis of *Acanthamoeba* keratitis (AK) (Peretz et al., 2015). Heredero-Bermejo et al., observed that both the BHI medium (brain-heart infusion, standard medium in microbiology, rarely used in amoeba culture) and the PYG prepared with Pancreatic Digest of Casein (Bacto casitone) are the most appropriate for growing and maintaining the *Acanthamoeba castellanii* strain. Also, PYG-Bacto casitone is the most suitable for conducting prolonged pharmacological screening assays prolonged (Heredero-Bermejo et al., 2012). In a study by Rahdar et al., while amoeba growth in PYG medium was not successful, efficient culturing in TYI-S-33 could be achieved. Therefore, they conclude that TYI-S-33 could be a substitute for PYG medium (Rahdar et al., 2012). PYG and NNA media are used daily by researchers working with *Acanthamoeba*, both for the isolation and identification of *Acanthamoeba spp.*, in addition to its maintenance over many years (Milanez et al., 2020; Wopereis et al., 2020; Retana et al., 2020). Few studies have been conducted comparing *Acanthamoeba* growth in different culture media. As discussed above, newer and more accessible culture media can be tested for this amoeba.

5. Conclusion

The present investigation showed the advantages of using RPMI-FBS medium to increase the growth of *Acanthamoeba* in axenic culture media. Furthermore, *Acanthamoeba* was observed to grow faster in nutrient-free agar (NNA) culture medium supplemented with RPMI-FBS compared to the other media and the usual method of adding bacteria.

Declarations

Author contribution statement

Alireza Latiﬁ: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
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