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Original Article

Comparative study of three xenic media culture for cultivation of *Balantidium coli* strains

Estudo comparativo entre três meios de cultivo xênicos para o cultivo de cepas de *Balantidium coli*

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Abstract

The aim of the present study was to evaluate the growth rate of *Balantidium coli* in three xenic media cultures. Between 2013 and 2015, 10 *B. coli* isolates obtained from feces of Cynomolgus macaques, and 30 isolates from feces of pigs were studied. An inoculum of 500 trophozoites was transferred to tubes containing LES, TYSGM-9 and Pavlova media. These cultures were evaluated at incubation times of 24, 48, 72 and 96 hours. In most of strains analyzed wasn't showed significant difference in the growth rate comparing TYSGM-9 and Pavlova media (Wilcoxon p>0.016). In Pavlova medium, the trophozoites showed a maximum growth at 72 hours with significant difference when compared with the times of 24 h and 96 h (Wilcoxon <0.008). In LES, viable trophozoites were observed until 24 hours, with a significant difference (Friedman p<0.05, Wilcoxon p<0.016) in the number of parasite cells compared with Pavlova and TYSGM-9 media cultures. Thus, LES medium seemed to be less adequate than the other media for maintenance of *B. coli*. Despite the satisfactory results in TYSGM-9, Pavlova medium was considered ideal for the maintenance of this protozoan strain, guaranteeing the viability of the parasite with subculture every three days, presenting lower costs.

Keywords: Protozoa intestinal, xenic culture, modified Pavlova, TYSGM-9, LES.

Resumo

O objetivo do presente estudo foi avaliar a taxa de crescimento de *Balantidium coli* em três meios de cultura xênicos. Entre 2013 e 2015 foram estudados 10 isolados de *B. coli* obtidos de Cynomolgus macaques e 30 isolados de suínos. Um inóculo contendo 500 trofozoítos foi transferido para tubos contendo os meios LES, TYSGM-9 e Pavlova. Os cultivos foram avaliados com tempos de incubação de 24, 48, 72 e 96 horas. Na maioria das cepas analisadas não foi observado diferença significativa na taxa de crescimento comparando TYSGM-9 e Pavlova (Wilcoxon p>0,016). Em Pavlova, os trofozoítos apresentaram máximo de crescimento a 72 h com diferença significativa quando se comparou com os tempos de 24 h e 96 h (Wilcoxon <0,008). Em LES observou-se trofozoítos viáveis até 24 horas com diferença significativa (Friedman p<0,05 e Wilcoxon p<0,016), na quantidade de células parasitárias, quando comparado com Pavlova e TYSGM-9. Dessa forma, o meio LES mostrou-se ser menos adequado do que os outros, para a manutenção de *B. coli*. Apesar do resultado satisfatório em TYSGM-9, Pavlova foi considerado ideal para manutenção do protozoário, por garantir a viabilidade do parasito com subcultivos a cada três dias, além de apresentar menor custo.

Palavras-chave: Protozoário intestinal, cultivo xênico, Pavlova modificado, TYSGM-9, LES.

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Introduction

Balantidium coli is an intestinal protozoan parasite primarily transmitted through the fecal-oral route, whereby the cysts are ingested in contaminated food and water (THOMPSON & SMITH, 2011). Balantidiasis has been reported in various animals, particularly non-human primates and pigs, which are considered the primary hosts of these parasites (NAKAUCHI, 1999; SCHUSTER & RAMIREZ-ÁVILA, 2008). This parasitic disease, considered a zoonosis, might manifest serious clinical gastrointestinal conditions in humans, such as dysentery and ulcers that progress to death (PINHEIRO & LIMA, 1991; ESTEBAN et al., 1998; VÁSQUEZ & VIDAL, 1999). This protozoan shows a wide geographical distribution and has primarily been reported in rural areas of developing countries with a tropical climate (ZAMAN, 1978; SOLAYMANI-MOHAMMADI & PETRI, 2006).

The first report of the xenic culture of *B. coli* occurred in 1921, demonstrating the use of a simple culture medium comprising saline and human serum (BARRETT & YARBROUGH, 1921). Over the years, several authors have isolated and maintained this protozoan in different culture media using different protocols. The culture media typically used for the isolation and maintenance of *B. coli* are those standardized for amebae, particularly *Entamoeba histolytica*, such as Boeck and Drbohlav medium (CUNHA & MUNIZ, 1937) and Pavlova xenic medium modified by Jones (CARNERI, 1972; BARBOSA et al., 2015). The maintenance of *B. coli* using monophasic culture medium containing gastric mucin has also been reported (KLAAS, 1974). Mucin is also incorporated in TYSGM-9 medium, which has been proposed for the cultivation of several species of amebae (DIAMOND, 1982). Although success in maintaining *B. coli* in xenic culture has previously been reported, there are no comparative studies of the efficiency of these culture media for the maintenance of this protozoan. Because *B. coli* is a parasite that has not been studied, it is not yet known which of these culture media could be more suitable for the maintenance of this protozoan in vitro.

It should be noted, xenic culture enables the maintenance of living protozoa. Furthermore, the culture of protozoa is considered an important feature that promotes and stimulates other studies, such as metabolism, immunodiagnostic, ultrastructure, and molecular studies, and the evaluation of drugs and experimental infection, which often require a large number of cells (CLARK & DIAMOND, 2002).

In this context, the aim of the present study was to evaluate the growth rate of *B. coli* strains isolated from pigs and non-human primates in media Pavlova (1938) modified by Jones (1946), and TYSGM-9 (DIAMOND, 1982) and Locke-Egg-Serum (BOECK & DRBOHLAV, 1925) under different incubation times.

Materials and Methods

Ethical considerations

This study design was approved through the Animal Ethics (CEUA-Fiocruz) Committee, license nº LW57/12, Protocol79P/11-2, annually SISBIO – IBAMA updated, under protocol number 31900-2.

Parasites

From August 2013 to September 2015, the growth rate of 40 strains of *Balantidium coli* in culture media was evaluated. These strains were maintained in the Laboratory of Protozoa in Cultivation (LAPAC) at the Institute Biomedical of the Federal University Fluminense in Pavlova culture medium (1938) modified by Jones (1946). The maintenance of *B. coli* strains is routinely carried out, with passages (subcultures) to new and fresh culture media, at 48 hours intervals. However, before of the passages, the culture medium receives a drop of rice starch suspension at 10%.

Among the 40 strains evaluated, 10 strains were isolated from fecal samples of non-human primates of the species *Macaca fascicularis* commonly known as Cynomolgus macaques, captive at the breeding center laboratory (Cecal) Fiocruz in Rio de Janeiro, and 30 strains were isolated from pig (*Sus scrofa domesticus*) feces obtained from different farms in the state of Rio de Janeiro.

Preparation of the culture media

The analyzed culture media were prepared according to the descriptions of their respective authors, Pavlova (1938) modified by Jones (1946); TYSGM-9 according to Diamond (1982); and LES (Locke-Egg-Serum) according to Boeck & Drbohlav (1925).

All media were sterilized through autoclaving at 120 °C for 20 minutes. After sterilization, 1 mL of antibiotic solution, containing streptomycin sulfate at a final concentration of 500 µg/mL, penicillin G potassium at a final concentration of 1000 IU/ml and 5% sterile and inactivated fetal bovine serum, was added. The culture media were distributed into sterile glass tubes (20 mm × 150 mm) with a screw cap. For the final assembly, after the addition of antibiotics and serum, the solid LES medium was covered with 8 mL of Locke solution. For the liquid monophasic media, modified Pavlova and TYSGM-9 were distributed into the glass tubes at final volumes of 10 and 8 mL, respectively.

Parasitic mass production

To produce the inoculum, trophozoites of the parasite were subjected to the quantitative expansion stage, i.e., increasing the number of parasitic cells. To this end, successive passes at 24-hour intervals were performed for three consecutive days, thereby increasing the number of subcultures, thus ensuring the inoculum required for all tubes. Each subculture strain received 60 µL of sterile rice starch suspension.

Production of the inoculum

The inoculum was produced from 1.5 mL of the sediment (rice starch precipitated in the tube) of the various cultivation tubes containing modified Pavlova culture medium. The mass of the various tubes was pooled in a 50-mL sterile centrifuge tube, and 1 mL of this inoculum was removed and transferred to a microtube. This material was homogenized, and subsequently 50 µL of this parasite suspension was transferred to another microtube, followed by the addition of 5 µL of buffered formalin

solution at 2%, generating 55 µL of solution. This volume was divided onto two microscope slides (depositing 27.5 µL onto each slide), subsequently covered with 24 mm × 32 mm coverslips, and read to count the trophozoites. The count was repeated after producing another two microscope slides (27.5 µL on each slide). After counting the number of trophozoites per mL, the required volume for the production of the standard inoculum of 500 parasitic cells was estimated.

An aliquot of the standard inoculum of 500 parasitic cells was placed in duplicate in 20 mm × 150 mm glass tubes with screw caps containing the respective culture media. At the time of inoculation, a drop of rice starch suspension was added to each medium (Figure 1). After inoculation, the tubes were incubated in a bacteriological incubator at 36 °C.

Analysis of the growth rate of B. coli

After 24, 48, 72 and 96 hours of incubation, 1-mL aliquots of the culture were removed from the bottom of the glass tube containing TYSGM-9, LES and modified Pavlova media and transferred to microtubes. After homogenization, 50 µL of the solutions were transferred to another microtube, to which was added 5 µL of solution-buffered formalin at 2%, totaling 55 µL. This volume was divided onto two microscope slides, each receiving 27.5 µL of the culture solution containing formalin and covered with 24 mm × 32 mm coverslips for subsequent counting. The number of trophozoites per mL was estimated in each medium. The count was performed in duplicate to guarantee the results.

Analysis of the results

The growth of the strains was evaluated based on an estimated amount of trophozoites present in each glass tube under different incubation times. The strains were considered at the quantitative growth (log phase) stage when the amount of trophozoites exceeded the inoculum (500 trophozoites/mL). The strains were considered under maintenance when a negative count was obtained. To compare the significant quantitative growth of *B. coli* trophozoites in the three xenic media culture at four incubation times, the entire experiment was repeated at different periods. The data obtained after counting the trophozoite strains from the pigs and Cynomolgus macaques were analyzed using Friedman and Wilcoxon non-parametric tests because these didn't present normal statistical distribution. These tests were performed with SPSS statistical software version 17 (SPSS®, Chicago, IL). In the Friedman test was used with a significance level of 5% to verify if there was difference in the growth of the protozoa in the culture media and at different incubation times. Wilcoxon test was used to compare the growth of *B. coli* between culture media analyzing the different incubation times. In this test the significance level of 5% were equally divided between all comparisons (modified Pavlova versus TYSGM-9, LES versus modified Pavlova and LES versus TYSGM-9), thus 5% was divided by three groups obtaining significance level 0.016. Analysis of *B. coli* growth according to the incubation time was performed by comparing the times in each culture medium used. Six groups were obtained (24 h versus 48 h, 24 h versus 72 h, 24 h versus 96 h, 48 h versus 72 h, 48 h versus 96 h, 72 h versus 96 h). The level of significance for this analysis was then 5% divided by six, being 0.008.

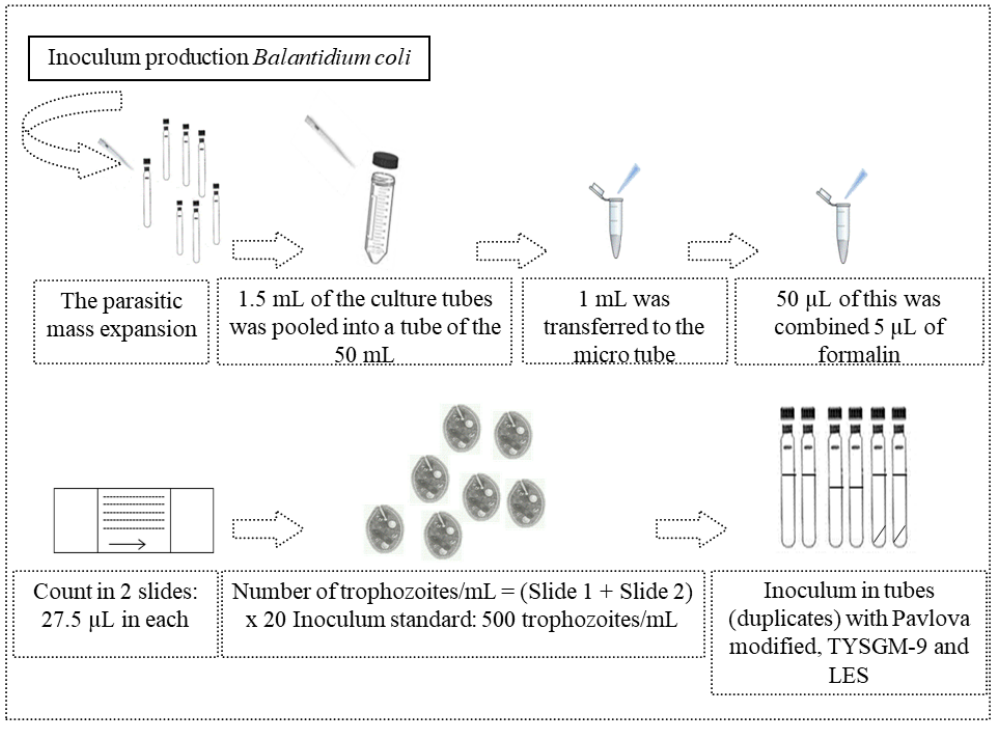

Figure 1. Flow chart of the inoculum production.

Results

The *B. coli* strains isolated from Cynomolgus macaques and pigs maintained in TYSGM-9 medium showed quantitative growth from inoculation until 48 hours of incubation (Figure 2). In the modified Pavlova medium quantitative growth was observed after 24 hours, and the maximum counts were obtained after 72 hours (Figure 2; Table 1). After these periods, the trophozoite number declined in both media. Similar to TYSGM-9, the modified Pavlova medium maintained *B. coli* strains isolated from Cynomolgus macaques and pigs after 72 hours of incubation (Table 1).

The comparison of *B. coli* growth in the culture media analyzed in Friedman test showed p-value<0.05 (p = 0.000), demonstrating that there was some difference in the quantitative growth of the protozoan in the culture media. In the LES medium, the trophozoites showed no significant growth from the inoculum. The difference in protozoan growth was evidenced when the performance of the LES medium was compared with the other two culture media, modified Pavlova and TYSGM-9. In the Wilcoxon test, at all incubation times the comparison of the other media with LES showed a p-value <0.016 (Table 1).

The highest trophozoites counts per mL were observed after 48 and 72 hours of incubation in TYSGM-9 media, showing strains isolated from Cynomolgus macaques with over 10,000 trophozoites per mL. In pig isolates, the largest parasitic cell count was observed at 48, 72 and 96 hours of incubation in TYSGM-9 medium, peaking

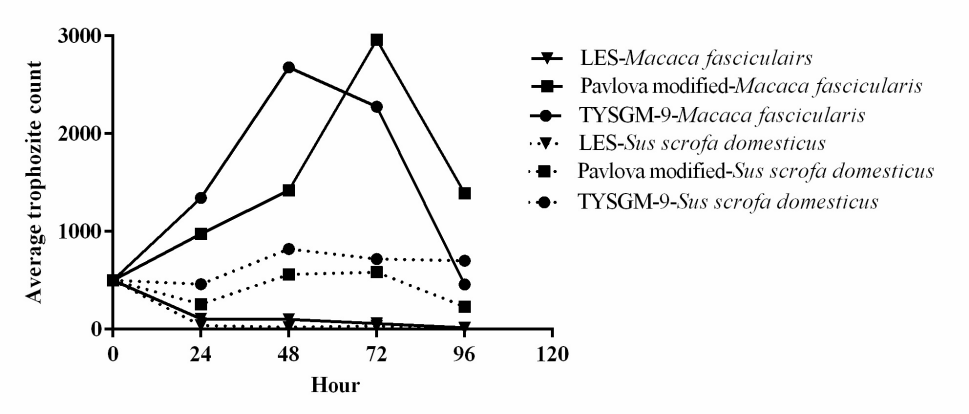

Figure 2. Quantitative growth pattern of *Balantidium coli* trophozoites isolated from Cynomolgus macaques and pigs and maintained at 24, 48, 72 and 96 hours of incubation in modified Pavlova, TYSGM-9 and LES culture media.

Table 1. Viable *Balantidium coli* trophozoite counts after 24, 48, 72 and 96 hours of incubation.

Culture media	Median values and number minimum and maximum of trophozoites			
	24 h (x 10^2)	48 h (x 10^2)	72 h (x 10^2)	96 h (x 10^2)
Strains *B. coli* of Cynomolgus macaques				
Modified Pavlova	7.97	11.7	9.3	2.45
	(1.8 ¬ 40)	(2.64 ¬ 35)	(1.15 ¬ 113.45)	(0.15 ¬ 59.5)
TYSGM9	5.52	9.63	4.77	3.07
	(1 ¬ 64.4)	(1.65 ¬ 128.95)	(1.27 ¬ 165.75)	(0.07 ¬ 14.9)
LES	0.22	0.37	0.1	0.05
	(0.05 ¬ 5.85)	(0.05 ¬ 4.3)	(0 ¬ 3.1)	(0 ¬1.15)
Wilcoxon test Modified Pavlova X TYSGM9	p=0.922	p=0.922	p=0.695	p=0.432
Wilcoxon test Modified Pavlova X LES	p=0.002*	p=0.002*	p=0.002*	p=0.002*
Wilcoxon test TYSGM9 X LES	p=0.002*	p=0.002*	p=0.002*	p=0.008*
Strains *B. coli* of pigs				
Modified Pavlova	1.95	4.08	3.13	0.45
	(0.02 ¬ 13.85)	(0.15 ¬ 25.92)	(0.02 ¬ 26.7)	(0 ¬ 20.65)
TYSGM9	2.06	5.56	4.62	1.31
	(0.01 ¬ 47.3)	(0.2 ¬ 66.55)	(0.25 ¬ 24.02)	(0 ¬ 33.75)
LES	0.06	0.75	0.02	0
	(0.01 ¬ 6.5)	(0 ¬ 1.2)	(0 ¬ 5.9)	(0 ¬ 0.92)
Wilcoxon test Modified Pavlova X TYSGM9	p=0.235	p=0.128	p=0.260	p=0.008*
Wilcoxon test Modified Pavlova X LES	p=0.000*	p=0.000*	p=0.000*	p=0.000*
Wilcoxon test TYSGM9 X LES	p=0.000*	0.000*	p=0.000*	p=0.000*

at more than 6,000 trophozoites at 48 hours of incubation. There was no significant variation in the trophozoite counts between the TYSGM-9 and modified Pavlova culture media (p> 0.016) in the Wilcoxon test, except in the trophozoite with 96 hours of *B. coli* from pigs. LES presented a significant variation in the amount of *B. coli* compared with both modified Pavlova and TYSGM-9 media. In addition, LES ensured the maintenance of strains for only up to 24 hours (Figure 2; Table 1).

In general, the log phase for growing strains of *B. coli* in TYSGM-9 medium occurred from 24 to 48 hours, whereas in modified Pavlova media, the log phase occurred from 24 to 72 hours, for trophozoites isolated from both Cynomolgus macaques and pigs. The stationary phase was short or nearly absent and decreased in TYSGM-9 and modified Pavlova media after 48 and 72 hours of incubation, respectively (Figure 2).

The variation of the protozoan growth in the different incubation times in each culture medium can be confirmed by the Friedman test, p <0.05. In the Cynomolgus macaques and pig strains p-values in the Friedman test were, respectively, Pavlova modified p = 0.009 and p = 0.000, in TYSGM9 p = 0.030 and p = 0.000 and in LES p = 0.003 and p = 0.000. The Wilcoxon test can demonstrate where this difference was occurring, p <0.008. In macaques isolates the growth difference occurred in the comparisons of 72 × 96 h times in modified Pavlova and 48 x 96 h in TYSGM-9. In the isolated strains of pig feces in the modified Pavlova medium and TYSGM9, the significant growth difference was observed between 24h x 48h and 24h x 72h times. In LES in both *B. coli* strains of pigs and Cynomolgus macaques strains a significant difference was observed when the incubation times were compared 24 × 96 h and 48 × 96 h (Table 2).

Discussion

The growth rate of *B. coli* in modified Pavlova, TYSGM-9 and LES media was evaluated at four incubation times: 24, 48, 72 and 96 hours, where 24 and 48 hours have been the most used in the maintenance of this ciliated parasite (BARRETT & YARBROUGH, 1921; CUNHA & MUNIZ, 1937; SVENSSON, 1955; ZAMAN, 1964). However, Cox (1961) successfully maintained these protozoan strains after 72 hours of incubation. In the present study, the media were evaluated after counting the trophozoites. Due to the large dimensions of the *B. coli* trophozoites, the counts were performed with an aliquot subdivided onto two microscope slides. Notably, many ciliated parasitic cells are lost when performing this assessment using a Neubauer chamber.

TYSGM-9 presented as a xenic system suitable for the quantitative expansion and maintenance of *B. coli* trophozoites in vitro, as strains isolated from Cynomolgus macaques and pigs showed growth when inoculated into this medium, characterized in the log phase at 48 hours after incubation. Subsequently, the number of cells declined. In general, the trophozoites of pigs maintained viability for up to 72 hours and some strains were maintained until 96 hours, while those of Cynomolgus macaques maintained viability for up to 96 hours. This degree of maintenance indicated the need for the frequent transfer of these strains to fresh media every three days. The interval time to transfer the strains facilitates programming of the transfers and production of the culture medium, thus ensuring the increased productivity of the laboratory.

According to Diamond (1982), culture media, such as TYSGM-9, which contains gastric mucin, are satisfactory for the cultivation of various intestinal parasites. Klaas (1974) reported similar results after successfully maintaining *B. coli* strains obtained from pigs, passing every three days and using xenic medium containing gastric mucin. Dolkart & Halpern (1958) reported that gastric mucin contains substances that promote the growth of intestinal protozoa. Thus, gastric mucin is a natural component of the intestinal mucosa, a common habitat of this parasite. Furthermore, TYSGM-9 was the culture medium showing the most significant quantitative development of the strains, particularly those isolated from pigs. The porcine gastric mucin used in the present study might have improved the development of strains isolated from pigs, as mucin is a specific component of the digestive system of the hosts. This may have favored the statistical difference observed in the Wilcoxon test in the trophozoites of pigs at 96 hours of incubation when compared TYSGM-9 and modified Pavlova.

TYSGM-9 was ideal for studies requiring fast parasitic mass because this medium presented the maximum trophozoites in less time (48 hours) and high counts of trophozoites per milliliter could be observed for strains isolated from both animal species. These results are consistent with those of Diamond (1982), who also reported high growth after 48 hours using this medium for the cultivation of *Entamoeba histolytica*, *Entamoeba coli*, *Entamoeba gingivalis* and *Dientamoeba fragilis* from the inoculation of 5,000 trophozoites per milliliter.

Table 2. Comparison of *Balantidium coli* incubation times in xenic culture media.

Culture media	Incubation time and p-value of Wilcoxon test					
	24 × 48 h	24 × 72 h	24 × 96 h	48 × 72 h	48 × 96 h	72 × 96 h
Strains *B. coli* of Cynomolgus macaques						
Modified Pavlova	p=0.084	p=0.193	p=0.846	p=0.625	p=0.432	p=0.002*
TYSGM9	p=0.064	p=0.770	p=0.244	p=0.193	p=0.006*	p=0.275
LES	p=0.797	p=0.287	p=0.008*	p=0.098	p=0.004*	p=0.016
Strains *B. coli* of pigs						
Modified Pavlova	p=0.000*	p=0.001*	p=0.087	p=0.851	p=0.001*	p=0.000*
TYSGM9	p=0.000*	p=0.005*	p=0.491	p=0.730	p=0.452	p=0.058
LES	p=0.665	p=0.127	p=0.000*	p=0.129	p=0.001*	p=0.006*

Wilcoxon Test p <0.008; * = p significant.

Similar to TYSGM-9, modified Pavlova medium maintained *B. coli* strains isolated from Cynomolgus macaques and pigs at 72 hours of incubation and also highlighted the need for passages to fresh media every three days. Lima & Hirschfeld (1996) and Silva (1997) also reported the successful maintenance with strains of *E. histolytica* in modified Pavlova medium with subculture at 72-hour intervals. Although the effects of the subculture times were not examined, Carneri (1972) reported the use of this medium for the cultivation of *B. coli* strains isolated from pigs for drug screening studies. Notably, only monophasic media were used in this study, including TYSGM -9 and modified Pavlova media, containing yeast extract. This substance is a major stimulator of growth in xenic cultures and can be an excellent supplier of nucleic acids.

In modified Pavlova medium, high counts of trophozoite isolates from Cynomolgus macaques were also observed, obtaining a maximum level of cells at 72 hours of incubation, demonstrating slow multiplication compared with the maintenance of such strains in TYSGM-9 media. According to Cox (1961), the strains that quickly achieve the highest level of growth will deplete culture medium nutrients and consequently present an early decline phase. This phenomenon was observed with *B. coli* strains cultivated in TYSGM-9 medium. Furthermore, in both modified Pavlova and TYSGM-9, the moderate growth of pig strains was observed, although these strains showed viability after 72 hours of incubation.

It should be noted that in this study a greater number of parasite cells were detected by counting in the incubation times of 48 hours and 72 hours, highlighting the efficiency of the incubation period at 48 hours for TYSGM-9 and 72 hours for modified Pavlova. This fact seems to have favored the significant difference verified in the Wilcoxon test, when comparing these times with those that presented the least amount of parasite cells, which was the first time of analysis, 24 hours, where the trophozoites were still adapting to the media and beginning to expand and the last time of analysis was 96 hours, in which the parasite cells were already dying and thus decreasing in quantity.

LES has successfully been used for the isolation and in vitro maintenance of *B. coli* strains isolated from pig, humans and non-human primates (CUNHA & MUNIZ, 1937). However, in the present study, this medium was not a xenic culture system suitable for the in vitro maintenance of the trophozoites isolated from Cynomolgus macaques and pigs. LES ensured the maintenance of trophozoites for only 24 hours. Such xenic media are less frequently utilized because their preparation is laborious and time-consuming (DIAMOND, 1982). To be biphasic, LES requires more than one day for preparation, including tilting and hardening the solid portion; autoclaving the cover solution (Locke's solution) and solid portion and mounting the tubes containing the solid bezel with the liquid portion, comprising Locke solution supplemented with serum.

Modified Pavlova and TYSGM-9 media present simplified production and easier assembly, as these solutions are liquid-phase media, requiring only one day for preparation. Additionally, these monophasic liquid media can be maintained for a month or more at refrigerator temperature (4 °C), while LES cannot be stored for a long time, as it has a solid phase comprising chicken eggs, which tend to dry and crack when stored for long periods.

In the present study, the unfavorable performance of LES medium compared with other media primarily reflects the lack of nutrients necessary for the maintenance of the metabolism of ciliates. The solid surface beveled format of LES produced using chicken eggs might also show a negative effect on the maintenance of *B. coli* strains. Ciliates and rice starch grains, a carbohydrate source, might stick to the bezel, hindering the movement of the parasites and the acquisition of food. Difficulties in the acquisition of the carbohydrate by the protozoan were not observed in the liquid-phase media examined: in both modified Pavlova and TYSGM-9, the heavy ciliates and rice starch grains tended to deposit on the tube bottom, facilitating parasite access to the food. In addition, Diamond (1954) pointed out that the LE culture medium allows the growth of bacteria with ease, discourage the multiplication of the protozoan, as the glucose used in Locke's solution can function as a substrate for bacterial development.

In general, the standard inoculum of 500 trophozoites of *B. coli* facilitates the estimation of the yield and the amount of culture medium. The isolated simian fecal material cultured in monophasic, modified Pavlova and TYSGM-9 media typically presents amounts equal to or greater than cultures inoculated with a single inoculum incubated up to 48 hours. While for trophozoites isolated from the fecal samples of pigs, the same amount can only be achieved with the preparation of two glass tubes containing a specific medium. This estimate was based on the measure of the central tendency and does not reflect extreme growth values (minimum and maximum), which were also observed in the present study. According to Diamond (1954) and Schuster & Ramirez-Ávila (2008), the strains might behave differently in vitro, even when isolated from a single host. The vast majority of strains isolated from Cynomolgus macaques presented more cells than those isolated from pigs, and distinct quantitative growth differences were observed between strains of the same species of animals. Studies using in vitro protozoa cultures should consider this diversity of growth, as it is extremely important to conduct the counting steps.

The assessment of the maintenance of these strains was observed using a growth curve considering the average values obtained. The difference in growth of the isolates made it difficult to select a culture medium optimal for all strains. Therefore, for maintaining protozoa, a xenic medium culture should be used, with the longest possible incubation time interval to ensure the viability of all strains.

The results obtained in the present study highlighted the importance of liquid monophasic xenic media, TYSGM-9 and modified Pavlova, emphasizing the use of both media to guarantee in vitro protozoan maintenance. Notably, the TYSGM-9 medium was the most suitable for studies requiring parasitic mass production. However, TYSGM-9 medium is still far from suitable for routine laboratory maintenance, as gastric mucin is not an easy reagent to obtain and presents a high cost. Nonetheless, when comparing the monophasic media studied, modified Pavlova media was considered ideal for maintaining the ciliate strains, ensuring the viability of the parasite for up to 72 hours of incubation and providing safe intervals for subcultures, representing an inexpensive and easy to prepare xenic system.

The results of the present study contribute information to the knowledge of *B. coli* growth when maintained in different xenic media, as outlined in the kinetic growth curve. This delineation enables the optimization of the laboratory routine and maintenance of ciliates in xenic culture through the programming of subcultures every 72 hours of incubation. The determination of the transfer intervals decreased the number of unnecessary subcultures, consequently reducing expenditures on the reagents used in the manufacture of media and enabling the reduction of the workload in the laboratory. The results also highlighted the need for further studies to evaluate *B. coli* growth kinetics, as there is little information about the in vitro maintenance of this ciliate, mainly in xenic culture systems.

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