AXENIC CULTURE AND CRYOPRESERVATION OF 

GIARDIA LAMBLIA ISOLATED IN IRAN

M. REZAIAN AND S. M. GHALHARI*

From the Department of Parasitology, School of Public Health, Tehran University of Medical Sciences, and the *Department of Parasitology, Pasteur Institute, Tehran, Islamic Republic of Iran.

ABSTRACT

Giardia lamblia cysts were isolated from fecal samples of 55 symptomatic and asymptomatic patients with giardiasis. The cysts were harvested and purified using the sucrose gradient method. The excystation of G. lamblia was obtained by treating them with HCl (0.01N), then they were transferred on to modified TYI-S-33 medium. Out of 55 stool samples 17 cases were excysted with a rate of 4-32%, but we were able to cultivate only one case with 12% excystation in culture medium. The generation time of G. lamblia was calculated to be about 10.33 hours. Cryopreservation of trophozoites was successfully done in liquid nitrogen and the thawing process had a survival rate of 85-90%.

INTRODUCTION

Giardia lamblia is the first reported intestinal protozoan with a world-wide distribution.1420 Giardia infection may be completely symptom-free or associated with serious diarrhea leading to malabsorption. It produces more clinical manifestations in children than in adults. Since parasitological tests cannot detect more than 50%-75% of cases,210 researchers continue seeking a more accurate immunological test for its diagnosis. The basis of these techniques depends on availability of pure antigens in sufficient quantities. Axenic culture of giardia is the key to the production of these antigens.

Karapetian was the first to report successful axenic cultivation of G. lamblia, symbiotically with chick fibroblasts and Candida guilliermondii.7,13 Subsequently, in 1976 axenic culture of giardia by E.A. Meyer made it possible to investigate the different aspects of this parasite.14 In the present study our goal was to cultivate and maintain an Iranian strain of G. lamblia in the laboratory in order to produce pure antigens for use in immunodiagnostic assays and other research purposes.

MATERIALS AND METHOD

Purification Giardia lamblia cysts were isolated from the feces of patients with high cyst excretion.6 Stools were broken up in tap water and were filtered through two or three layers of cheese cloth gauze. 3 ml of the fecal suspension was layered on 3ml of 0.85 M sucrose and centrifuged at 600 g for 10 min at room temperature (preferably 4°C), then the cysts were collected from the layer between the sucrose-water phase. Washed cysts were carefully added on the top of a discontinuous density gradient consisting of two 3ml layers of 0.85 M and 0.4 M sucrose. After centrifugation at 600 g for 10 min, cysts concentrated at the 0.85-0.4M sucrose interface were collected and washed again. Purified cysts were resuspended in distilled water (D.W.) and stored at 4°C.

Excystation and Cultivation In this study we used the excystation procedure of Bingham and Meyer.1,13 Concentrated cysts were diluted 1:10 with HCl (0.01N). The preparation was incubated at 37°C for one hour and centrifuged at 600 g for 10 min. The supernatant was removed and...
Culture and Cryopreservation of *Giardia lamblia* Isolated in Iran

The pellet washed with D.W. and about $10^4$-$10^6$ cysts were inoculated into the 13x100 mm screw-capped borosilicate tube containing TYI-S-33 culture medium. The modified version of TYI-S-33 medium was used for the culture of *G. lamblia.* Each 100 ml of medium contained the following: 100mg K$_2$HPO$_4$, 60mg KH$_2$PO$_4$, 2 gr trypticase, 1.0 gr yeast extract, 1.0 gr glucose, 200 mg NaCl, 200 mg cystein-HCl monohydrate, 20mg ascorbic acid, 2.28mg ferric ammonium citrate, 50-100 mg dehydrated bovine bile and 10 ml of inactivated bovine serum (pH: 7.0-7.2). The medium was sterilized by passing it through a 0.45 μm membrane filter, and then supplemented with 15% bovine serum, penicillin (400 IU/ml), streptomycin (500 μg/ml), gentamicin (50 μg/ml) and if necessary amikacin (125 μg/ml). The prepared medium was dispensed into tubes filled to 80% capacity which could be used for 7-10 days at 4°C. The inoculated culture tubes were checked after one hour of incubation at 37°C for excystation and every 24 hours for development and division of the trophozoites with an inverted microscope. After the trophozoites developed, the medium was replaced every 2-4 days. When trophozoites grew well and formed a dense monolayer on the surface of the tube walls, they were dislodged by immersing the tubes in an ice-water bath for 10 min and shaking them for several seconds. 1-2 ml of this trophozoite suspension was inoculated into a tube of fresh medium.

**Cryopreservation**

For cryopreservation of *Giardia lamblia* trophozoites, dimethyl sulfoxide (DMSO) was used as a protectant. The trophozoites were suspended in TYI-S-33 medium containing 10% DMSO and distributed into screw-capped cryopreservation tubes and transferred to either a -70°C freezer or a liquid nitrogen tank.

**RESULTS**

The present study was performed on 55 fecal samples from patients with high cyst excretion. Out of 55 stool samples 26 cases had no excystation. Surprisingly, in 12 cases the cysts were observed without a cyst wall and the parasites were non-motile. Seventeen cases were found to be excysted with a rate of 4-32% (Table I).

Except for 1 case, the rest of the samples with excysted parasites were found to harbor head trophozoites after 1-7 days. One of these two cultures was discarded because of a

<table>
<thead>
<tr>
<th>Number of fecal samples</th>
<th>26</th>
<th>12</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excystation rate</td>
<td>0%</td>
<td>cysts without cyst wall</td>
<td>4-32%</td>
</tr>
</tbody>
</table>

Table I. Excystation rate of *G. lamblia* cysts recovered from fecal samples.

![Giardia lamblia trophozoites in culture medium (without staining).](image1)

Fig. 1.

![Giardia lamblia trophozoites derived from culture medium (Giemsa staining).](image2)

Fig. 2.

![Right: empty Giardia lamblia cysts, intact cysts, and an excysted trophozoite. Left: Longitudinal division of trophozoites.](image3)

Fig. 3.
Monolayer surface was seen 14 days after hatching. Keister reported that trophozoites in the logarithmic phase could attach to the wall of the culture tube with a rate of 80-90%, and our study showed an attaching rate of 70%.

After Karapetian, the founder of giardia cultivation, many scientists have since investigated culturing *Giardia lamblia* in different media such as HSP₁, HSP₂, TPS, and TYI-S-33. Kasprzak reported the generation time of 20 strains of *G. lamblia* to range from 8.1±0.2 to 31.2±2.6 in the TPS medium. This period decreased markedly (from 8.1±0.2 to 15.6±0.5) in BI-S-33 supplemented with bovine bile. Addition of bovine bile to TYI-S-33 medium also reduced the generation time. The lowest generation time was obtained with fresh bovine bile (7.5±0.5 hours). Lu et al. reported the generation time in the TYI-S-33 medium to be 15±0.5 hours. In our study the calculated generation time was about 10.33 hours.

After reviewing the literature and our own results, we believe that *G. lamblia* is a very fastidious organism with an excystation rate, generation time and media adaptation which is related to differences between strains. Therefore, the development of defined medium without undefined biological products is needed to advance the axenic culture systems of giardia.

**REFERENCES**

6- Hautus MA, Kortbeek LM: In vitro excystation and subsequent...
Culture and Cryopreservation of *Giardia lamblia* Isolated in Iran


