The Korean Journal of Parasitology
Vol. 33, No. 4, 387-390, December 1995

☐ **Brief Communication** ☐

Axenization of *Entamoeba histolytica*, a Korean strain YS-27

Jae-Kyung CHANG, Kyung-il IM* and Chin-Thack SOH

Institute of Tropical Medicine & Department of Parasitology, Yonsei University College of Medicine Seoul 120-752, Korea

Abstract: In the present communication, YS-27, a Korean strain of *Entamoeba histolytica* is described for the isolation and establishment of axenic cultivation. *E. histolytica*, designated as strain "YS-27", was isolated from the pus of a hepatic abscess obtained from a 72 year old inpatient of August 10, 1969. Specimens, were obtained by needle aspiration, inoculated immediately and weekly cultured in a modified diphasic medium at 37°C. Strain YS-27 had been maintained for more than 15 years by weekly subculture until February, 1985. These cultures were transferred to a monoxenic TTY-SB medium seeded with a trypanosomatid of the genus *Crithidia*. Penicillin G, 2 to 10 × 10^3 International units and Streptomycin, 2 to 10 mg per 100 ml, were added to the cultures to eliminate the bacteria. After more than one year later, these two organisms were well maintained by transfer every 3 or 4 days until January, 1986 at 37°C in TTY-SB medium in the absence of other microorganisms. These monoxenic cultures were then transferred to TYI-S-33 medium. Strain YS-27 alone had not been growing at the time of transfer, but when overlaid with *Crithidia* at intervals of 3 to 4 days, strain YS-27 propagated well. The *Crithidia* died out several weeks later after several passages. Beginning in April, 1986, strain YS-27, was successfully established in axenic culture in TYI-S-33 medium and has been maintained in continuous culture and multiplied well to present.

Key words: *Entamoeba histolytica*, Korean strain, YS-27, axenization

Entamoeba histolytica has been cultivated *in vitro* since 1925 (Boeck *et al.*, 1925), but always in the presence of bacteria. Amoeba cultivation in the absence of bacteria is of extreme importance, since bacteria-free cultivation is a prerequisite for basic protozoan and amebiasis studies. Recently, several noticeable improvements in cultivation techniques have been developed, identifying essential growth requirements of this amoeba. Although there are many reports on the culture of *Entamoeba*, the first consistent and confirmed axenic results were reported by Stoll (1957). Subsequently, Diamond (1961) reported a clear monophasic medium which supported *E. histolytica* as well as a related species, *E. terrapinae*.

Cultures of *E. histolytica* are readily established in association with certain species of bacteria or trypanosomatids. Phillips (1950, 1951) was the first to cultivate *E. histolytica* serially in association with *Trypanosoma cruzi*. Phillips (1950, 1951) and Pan (1960) described practical methods for the monoxenic cultivation of *E. histolytica* with *T. cruzi* and other trypanosomatids. Pan (1960) reported the simple cultivation method of *E. histolytica* and *E. histolytica*-like amebae in association with either *Crithidia* sp. or *T. cruzi*. Four strains of *E. histolytica* and two strains of *E. histolytica*-like amebae have been cultivated utilizing this technique. Diamond (1961) described a procedure for axenic cultivation of

• Received Oct. 16 1995, accepted after revision Nov. 21 1995.

* Corresponding author

E. histolytica. Since this publication, the original diphasic medium has undergone several modifications, resulting in a simplified and improved medium which is particularly useful for initiation of axenic cultures.

In the present communication, the isolation and axenic cultivation of a Korean strain of *E. histolytica*, YS-27, is described.

A strain of *E. histolytica*, designated "YS-27", was isolated from the pus of a hepatic abscess obtained from a 72 year old patient (Kim, S.K.) on 10 August, 1969 hospitalized at Koryo Hospital, Seoul, Korea. The specimens, obtained by needle aspiration, were inoculated immediately and initially cultured in a modified diphasic medium (Faust & Russell, 1964) at 37°C. *E. histolytica* strain YS-27 has continued to propagate and has been maintained in our laboratory for more than 15 years by weekly subculture until February, 1985.

These cultures were transferred to a monoxenic TTY-SB medium (Diamond, 1968) in association with *Crithidia*. *Crithidia* were obtained from NIH, USA (Dr. Diamond, L.S.) and cultured in TTY-SB medium (Table 1) at 25°C for a year by subinoculating 1 to 5 × 10^6 trophozoites into 10 ml of medium at weekly intervals.

Table 1. Composition of TTY-SB medium for monoxenic cultivation of *Entamoeba histolytica*.

Ingredients	Amount
Tryptose (Difco)	10.0 g
Trypticase (BBL)	10.0 g
Yeast extract (BBL)	10.0 g
Glucose	5.0 g
L-cysteine monohydrochloride	1.0 g
Ascorbic acid	0.2 g
Sodium chloride	5.0 g
Potassium phosphate, monobasic	0.8 g
Potassium phosphate, dibasic, anhydrous	0.8 g
Water, glass distilled, to make	950.0 ml
pH adjusted to 7.2 with 1N NaOH	
To complete the medium, add	
Horse serum	50.0 ml
Defibrinated rabbit blood	2.5 ml

*Ten ml aliquots of the medium were stored in screw-capped tubes at –20°C for several months until use.

E. histolytica, strain YS-27, was introduced into a monoxenic TTY-SB medium seeded with a trypanosomatid of the genus *Crithidia*. Penicillin G, 2 to 10 × 10^3 International units and Streptomycin, 2 to 10 mg per 100 ml, were added to the cultures to eliminate the bacteria growth. After more than one year later, these two organisms were maintained in the absence of other microorganisms at 37°C in TTY-SB medium by subinoculation every 3 to 4 days until January, 1986.

After establishment of monoxenic cultures, *E. histolytica*, strain YS-27, and *Crithidia* were transferred to the TYI-S-33 medium (Diamond *et al.*, 1978). At the time of transfer, strain YS-27 had not been growing alone, but when overlaid with *Crithidia* at intervals of 3 to 4 days, strain YS-27 propagated well. The *Crithidia* died out several weeks later after several passages. Beginning in April, 1986, strain YS-27 was successfully established in

Table 2. Composition of TYI-S-33 medium for axenic cultivation of *Entamoeba histolytica*

Ingredients	Amount
TYI broth	
Trypticase (BBL)	20.0 g
Yeast extract (BBL)	10.0 g
Glucose	10.0 g
Sodium chloride	2.0 g
Potassium phosphate, monbasic	0.6 g
Potassium phosphate, dibasic, anhydrous	1.0 g
L-cysteine monohydrochloride	1.0 g
L-ascorbic acid	200.0 mg
Ferric ammonium citrate	22.8 mg
Water, glass distilled, to make	870.0 ml
pH adjusted to 6.8 with 1N NaOH	
Filtered through Whatman No.1 filter paper and sterilized at 121°C for 15 minutes.	
To complete medium, add	
Bovine serum	100.0 ml
Vitamin mixture no. 13	20.0 ml

*TYI broth can be stored at –20°C for at least 6 months until use. Ten ml aliquots of TYI-S-33 media were stored in screw-capped tubes a maximum of one week prior to use. Vitamin mixture no. 13 is a modification of the mixture described by Diamond *et al.* (1978)

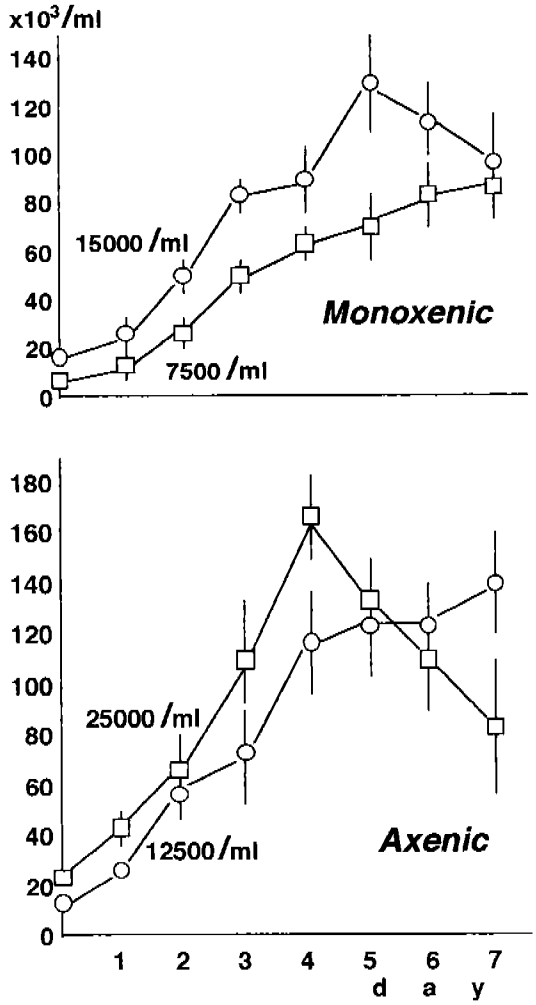

Fig.1. *In vitro* propagation of *Entamoeba histolytica* trophozoites in monoxenic TTY-SB medium (above) and in axenic TYI-S-33 medium.

axenic cultures in TYI-S-33 medium. Strain YS-27 has been maintained successfully in continuous culture and multiplied in the axenic state to present, October, 1995 (Fig.2). Peak growth in axenic culture occurred at day 4, with production of 6.8 times when the initial inoculum was 2.5×10^4 trophozoites, and the amoebic population decreased markedly after 4 days. When the initial inoculum was 1.25×10^4 trophozoites, the population continued to increase through day 7.

A method for axenic cultivation of *E. histolytica* was introduced by Diamond (1961). Since that time, improvements in culture media for continuous propagation have been made. Diamond (1968) reported the axenic cultivation of *E. histolytica*-like amebae for the first time. While established the axenization of *E. histolytica* using the TPS-1 medium, it failed to support continuous propagation of strain YS-27. Therefore, TYI-S-33 medium was used in the present study instead of TPS-1. Several strains of *E. histolytica* (200:NIH, HB-301:NIH, F-22, HK-9) and two strains of *E. histolytica*-like amebae (Huff and Laredo) had introduced that was growing well in axenic culture medium. This long term experiment demonstrates the establishment of amoebae from a hepatic abscess in axenic culture through the amoeba-bacterial stage and monoxenic cultures.

REFERENCES

Boeck WC, Drbohlav J (1925) The cultivation of *Endamoeba histolytica*. *Am J Hyg* **5:** 371-407.

Diamond LS (1961) Axenic cutivation of *Entamoeba histolytica*. *Science* **134:** 336-337.

Diamond LS (1968) Improved method for the monoxenic cultivation of *Entamoeba histolytica* Schaudinn, 1903 and *E. histolytica*-like amebae with trypanosomatids. *J Parasitol* **54:** 715-719.

Diamond LS, Harlow DR, Cunnick C (1978) A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*. *Trans Royal Soc Trop Med & Hyg* **72:** 431-432.

Faust EC, Russell PF (1964) Clinical Parasitology. 7th Ed. p. 994-997 Lea and Febiger

Pan CT (1960) Studies on the monoxenic cultivation of *Entamoeba histolytica* with hemoflagellates. *J Inf Dis* **106:** 284-289.

Phillips BP(1950) Cultivation of *Endamoeba histolytica* with *Trypanosoma cruzi*. *Science* **111:** 8-9.

Phillips BP (1951) Comparative effects of certain species of Trypnosomidae on the growth of *Endamoeba histolytica* in the absence of bacteria. *Am J Trop Med* **31:** 290-294.

Stoll NR (1957) Axenic serial culture in cell-free medium of *Entamoeba invadens*, a pathogenic amoeba of snakes. *Science* **126:** 1236.

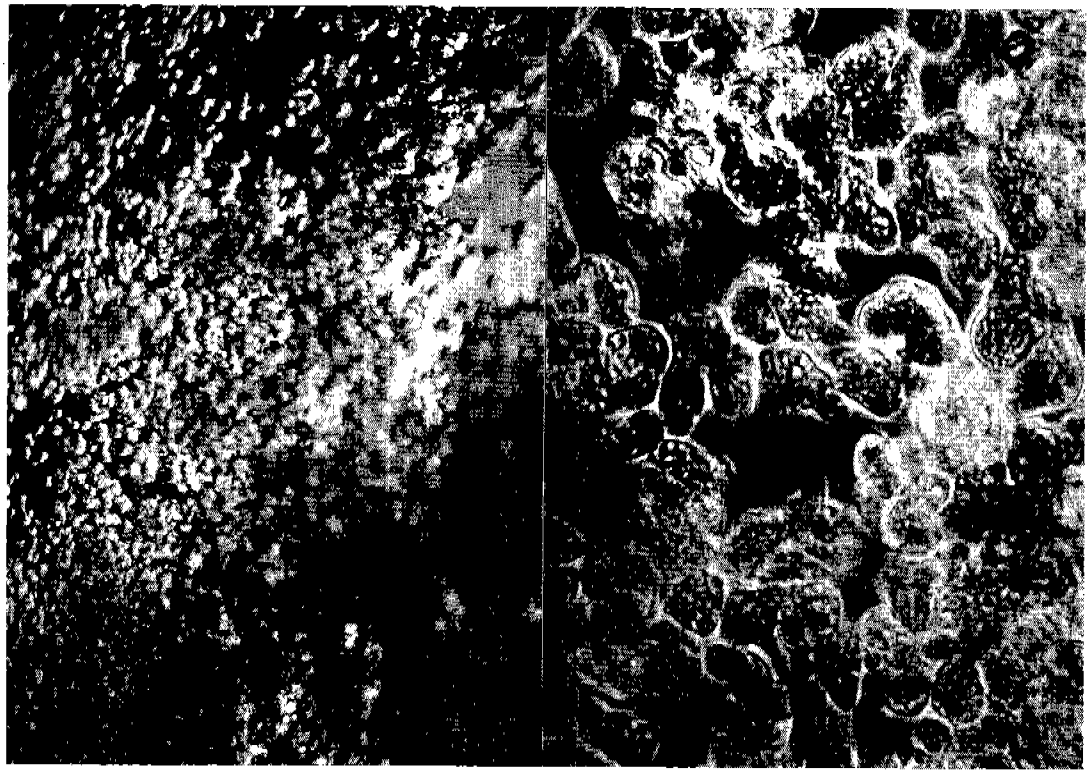

Fig. 2. 72-hours colonies of *Entamoeba histolytica*, strain YS-27, grown under axenic conditions in TYI-S-33 medium at 37°C (left, × 40; right, × 100).

=초록=

이질아메바 한국분리주 YS-27의 무균 배양화

장재경, 임경일, 소진탁

연세대학교 의과대학 열대의학연구소 및 기생충학교실

1969년 8월 10일 서울 고려병원의 입원환자 김○○의 간농양에서 채취한 고름을 modified diphasic배지(Faust and Russell, 1964)에서 배양하여 이질아메바 한국분리주 YS-27을 얻었다. 이를 1985년 2월부터 TTY-SB 배지에 넣어 *Crithidia*와 함께 37°C에서 혼합 배양하였으며 3-4일 간격으로 계대하였다. *Crithidia*는 Dr. L.S. Diamond(미, NIH)로 부터 분양받아 TTY-SB 배지 10 ml에 100-500만개씩 넣어 25°C에서 1주 간격으로 계대 배양하였다. Monoxenic 배지인 TTY-SB에서 이질아메바와 *Crithidia*를 혼합배양하면서 세균 제거를 위하여 배지 100 ml 당 Penicilin G 2,000-10,000 I.U.와 Streptomycin 2-10 mg을 첨가하였으며 세균이 완전히 제거되고 monoxenic 상태로 되기까지 1년 이상 걸렸다. TTY-SB 배지에서 axenic 배지 TYI-S-33으로 바꾸었더니 처음 이질아메바 영양형의 증식이 되지 않았으나 *Crithidia*를 넣어 주었더니 이질아메바의 증식이 잘 되었다. 3-4일 간격으로 계대 배양하였으며, 수주간 배양후에는 *Crithidia*를 보충하지 않아도 이질아메바영양형만 잘 증식 되었다. 이질아메바 한국분리주 YS-27은 1995년 10월 현재 TYI-S-33 배지에서 무균적으로 지속적으로 계대 배양되고 있으며, 영양형의 증식도 양호하다.

〔기생충학잡지 33(4): 387-390, 1995년 12월〕