A SIMPLE, RAPID AND REPRODUCIBLE TECHNIQUE FOR CLONAL CULTURE OF AXENICALLY GROWN *ENTAMOEBA HISTOLYTICA* AND OTHER *ENTAMOEBAE*

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A SIMPLE method has been described for the development of clonal growth of *E. histolytica* and other species of *Entamoebae*, such as Huff and Laredo strains and *E. invadens* (six strains).

Introduction

In the absence of clonal culture of *Entamoebae*, studies on the cell biology of these amoebae have been hampered very much in the past. No report, so far, is available for getting clonal cultures of axenically grown *E. histolytica*, originating from single trophozoite inoculated into the medium without incorporation of agar. The present communication describes for the first time the development of a simple technique for clonal cultures of axenic *Entamoebae*.

Materials and Methods

E. histolytica strains DKB, DKB-C (amoebae of DKB strain fed on cholesterol), IP-106; *E. histolytica*-like strains Huff and Laredo (JA) and *E. invadens* strains IP-1, IP-2, PZ, N, SiS, SiVL and BN; were used for cloning. All these strains were maintained in TYI-S-33 axenic medium developed by Diamond *et al.*[1]. *E. histolytica* strains were maintained at 37° C and *E. histolytica*-like amoebae and *E. invadens* at 25° C.

One drop of amoebae suspension from a 48 hr old culture tube was taken on a clean and sterile slide. The number of trophozoites per field of microscope was adjusted to 10 or 20 by dilution method, under the microscope placed inside a sterile laminar flow hood. Single motile trophozote was picked up by a fine capillary and transferred to the special perspex chamber (Fig. 1). The cavity of the chamber containing TY-IS-33 medium was sealed from the top by cover slip and molten wax, after trophozote was released inside. No air bubble was allowed to remain inside the chamber. The two openings, leading to the cavity, were also sealed with paraffin wax. The chamber was incubated at 37°/25° C. When a fairly good number of trophozoites developed the whole contents of the cavity were transferred to conventional screw-capped culture tubes containing fresh medium.

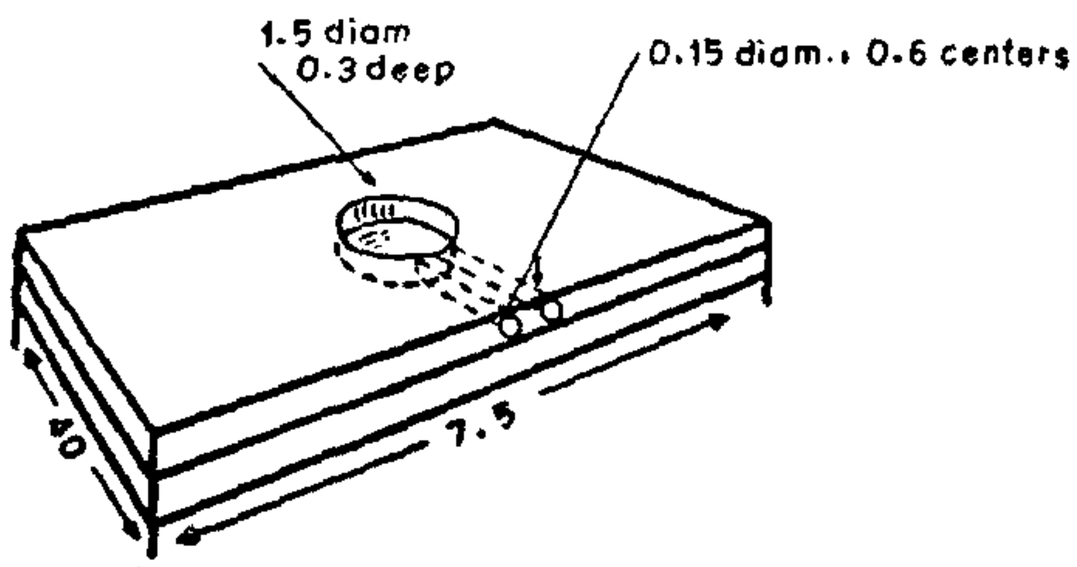

FIG. 1. "Perspex" perfusion chamber (all dimensions in centimeters).

Results

From Table I it can be seen that clone cultures developed from all the strains of *Entamoebae*. DKB, DKB-C and IP-106 gave rise to 60, 40 and 80% clone cultures respectively. *E. histolytica*-like strains Huff and Laredo (JA) and *E. invadens* IP-1, IP-2, PZ, BN, SiS and SiVL growing at 25° C showed clonal development of 100, 60, 80, 70, 75, 75, 75 and 80% respectively (Table 1), which were slightly more than *E. histolytica*. Bacterial contamination was observed in few cases which were discarded. Twenty single trophozoites were picked up for clonal culture in every strain. All the twenty single trophozoites of Huff strain gave rise to clonal cultures.

Discussion

Meerovitch[2] in his studies on growth of *E. invadens* in organo-typing cultures of embryonic chick intestine used "perspex" perfusion chambet (Fig. 1) which was made by cementing together two pieces of "perspex" one of which had the central culture chambe" and the two side holes drilled through it. Das[3] described a novel and rapid method for *in vitro* testing of anti-amoebic compounds by the use of cavity slides. Dubey and Das[4] and Das[5] used cavity slides for the growth of polyxenic cultures of *E. histolytica* for the studies on their nuclear division and clonal growth respectively. Gillian and Diamond[6] reported a technique fo. growing single *Entamoeba* trophozoite into colonies in agar and thereby, clonal growth of axenically grown *E. histolytica*. These workers used dilution method to get separated single trophozoite for forming colony in agar incorporated medium. It seems difficult to pick up a single colony inside the agar in a tube containing many colonies. Dutta[7] used dilution technique to get single trophozoite for clonal growth in test tubes. Picking up of a helathy motile trophozoite by a very fine capillary under the microscope, as described in this article, appears to be a more sound method of isolation of a single trophozoite for clonal growth. Low temperature strains of *Entamoebae* showed better rate of clonal growth as

TABLE I

Development of clonal cultures of different strains of Entamoeba *growing axenically in TYI-S-33 medium*

Strain of Entamoeba	No. of single trophozoite picked up	No. of culture established
	(growth temperature 37°)	
A. *E. histolytica*		
DKB	20	8
DKB–C*	20	12
P 106	20	16
	(growth temperature 25°)	
B. *E. histolytica*-like		
Huff	20	20
Laredo type-JA	20	12
C. *E. invadens*		
IP–1	20	16
IP–2	20	14
PZ	20	15
BN	20	15
SiS	20	15
SiVL	20	16

* Original DKB strain but trophozoites passaged through cholesterol upto twelve subcultures at every 72 hr.

compared to amoebae growing at 37° C. This finding is contrary to the findings of Gillian and Diamond[6] where they reported fewer microscopic colonies in low temperature strains. *E. histolytica* growing at 37° C are more fragile and susceptible to oxygen tension as compared to *E. histolytica*-like amoebae and *E. invadens* which grow at lower temperature and are more rigid to negative oxydation-reduction potential[8]. No marked and significant differences between "classical" *E. histolytica* strains and the non-pathogenic groups could be determined by the per cent development of clonal cultures as described by Gillian and Diamond[6].

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DERRONE-4′-O-METHYL ETHER FROM SEEDS OF DERRIS ROBUSTA

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WE report in this note, the isolation and characterization of the pyranoisoflavone, 4′-O-methyl derrone from the seeds of Derris robusta (fam : Leguminosae) during our investigation on this plant[1–7]. This isoflavone was reported earlier from *Calopogonium mucunoides*[8] by Vilain *et al.*[8].

The ethyl acetate and methanol extract of the seeds were found to be similar (TLC), hence combined and subjected to column chromatography over silica gel. Elution with benzene-ethyl acetate (9·3 : 0·7), gave a white crystalline compound (25 mg), m.p. 170–2°. It analysed for $C_{21}H_{18}O_5$, did not respond to Shinoda's test but gave a pink colour in Na/Hg-HCl reaction for isoflavonoids, a dark brown colour with alcoholic ferric chloride and a bright yellow colour with boric acid in acetic anhydride (Dimroth reagent[9]), characteristic of a strongly hydrogen bonded *ortho*-hydroxy carbonyl system. The latter is also supported by intense absorptions at 3350 (chelated hydroxyl) and 1650 (carbonyl) cm^{-1} in the IR spectrum and a strong signal at δ 12·59 in the NMR spectrum ($CDCl_3$) of the compound. An absorption band in UV at λ_{max}^{MeOH} 282 nm, which undergoes a bathochromic shift of 9 nm on addition of aluminium chloride suggested a 5-hydroxyisoflavone skeleton for the compound. That the compound is an isoflavone was confirmed by the sharp singlet in NMR at δ 7·75 (characteristic of H–2 of the isoflavones). Absence of a bathochromic shift with sodium acetate in the UV spectrum indicated the absence of a free hydroxyl at 7-position[10]. NMR of the compound also showed a six proton singlet at δ 1·38 due to *gem*-dimethyl group and two doublets ($J = 10$ Hz) at δ 6·55 and 5·45, each integrating for one proton, corresponding to vinylic protons (H-4″ and H-3″, respectively) thereby suggesting the presence of a 2,2-dimethylchromen