Table 1. Population of bacteria in endorhizosphere of different crops.

Crop	Bacterial population on TSA ($\times 10^6$/g root)	Phosphate-solubilizing bacteria ($\times 10^6$/g root)
Mango	37.92 ± 3.75	3.67 ± 0.25
Carrot	28.78 ± 5.01	7.25 ± 1.0
Maize	24.11 ± 4.01	7.05 ± 0.37
Beet root	22.50 ± 4.17	10.58 ± 0.75
Ragi	13.22 ± 1.79	4.15 ± 0.29
Sapota	10.36 ± 1.07	1.93 ± 0.14
Mulberry	7.15 ± 0.72	2.74 ± 0.6
Tomato	3.85 ± 0.77	0.23 ± 0.8
Cashew	2.50 ± 0.83	0.21 ± 0.4

± Indicates S.E.

Table 1 indicates that all plant roots harboured bacteria in the endorhizosphere to varying degrees. The highest population was observed in mango (3.79×10^7/g root) and the least in cashew (2.5×10^6). The present study also reveals that the phosphate-solubilizing bacteria are present in the endorhizosphere of the crop plants. They range from about 4% to 84% of the total endorhizosphere bacteria. Beet (*Beta vulgarts*), a non-mycorrhizal plant, harboured the highest number of phosphate-solubilizing bacteria. It is possible, therefore, that non-mycorrhizal plants may have higher population of phosphate solubilizers than mycorrhizal plants. Considerable work has already been done for studying the occurrence, distribution and ability of the bacterial isolates associated with crop plants particularly in the rhizosphere and soils[4]. However, to our knowledge no information is available on the presence of phosphate-solubilizing bacteria associated with endorhizosphere of any plant. Therefore, the information gathered in this study possibly opens a new area of research to understand the role of phosphate-solubilizing bacteria in the phosphorous nutrition of crop plants, because unlike rhizosphere and soil micro-organisms, the endorhizosphere micro-organisms are closely associated within the plants with greater degree of specificity.

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A simple method for clonal culture of *Entamoeba histolytica*

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A simple and reliable method was developed for clonal culture of *Entamoeba histolytica*. Strains of *E. histolytica* were isolated from different categories of amoebiasis cases and were maintained in Boeck and Drbohlav (B&D) medium. They were cloned in an overlay of B&D medium. Ten amoebae each were cloned from acute amoebic dysentery (AAD) and non-dysenteric amoebic colitis (NDAC) cases and six were cloned from amoebic liver abscess (ALA) cases. The success rate was 80, 60 and 83% for AAD, NDAC and ALA cases respectively. This method gives a better success rate than other methods of establishment of clones from different categories.

ANY culture of *Entamoeba histolytica* established *in vitro* will consists of heterogenous population of amoebae, i.e. they contain both pathogenic and non-pathogenic species. Even a single species may contain heterogenous population, e.g. NIH:200. The variation may be due to many factors like mutations, toxic substances secreted by amoebae and chemicals present in the medium. Therefore, the clones from indigenous strains of *E. histolytica* are desirable for immunological, biochemical, and genetic studies.

Reports are available for the clone cultures of *E. histolytica*[1-6]. Most of these workers cloned *E. histolytica* either from axenic cultures or with other species of Entamoebae. As far as we know only Farri[3] has isolated and cloned *E. histolytica* from different categories of amoebiasis. In the present report an attempt was made to initiate and maintain clone cultures of *E. histolytica* in Boeck and Drbohlav (B&D) and in liquid media.

B&D medium was prepared according to the method of Boeck and Drbohlave[7]. Liquid medium for clone cultures was prepared as follows: An overlay of B&D medium was pooled out in a screw-cap flat bottom flask after 24 h of incubation. It was mixed with inactivated bovine-serum in the ratio of 9:1 (9 parts pooled overlay + 1 part bovine serum).

The faecal samples positive for *E. histolytica* were incubated in B&D medium. These samples were taken from acute amoebic dysentry (AAD) and non-dysentric amoebic colitis (NDAC) cases and were subcultured regularly after every 48 h.

The clones were initiated from xenic cultures of B&D medium. A drop of profuse growth of xenic cultures was taken on a microscopic slide and was diluted with

normal saline in the ratio of 1:4. From this diluted sample a single amoeba was picked up (under low-power, 10X) using a micro-Pasteur pipette and was immediately put into the culture tubes containing 0.2 ml of medium. Likewise, amoebae were picked up from both the cases. Ten amoebae were picked up from each case.

Pus sample (about 0.5 ml) from amoebic liver abscess (ALA) was directly put into the 0.5 ml of liquid medium and all tubes were incubated at 37°C. After 48 h of incubation the tubes were centrifuged at 500 rpm for 5 min, the old medium was replaced by fresh medium and were again incubated at 37°C. These were regularly subcultured after every 48 h for 3–4 weeks thereafter the amoebae were transferred to culture tubes containing 0.5 ml of medium and were sub-cultured after every 96 h. By this time, the number of amoebae increased by 8–10 folds. Finally, the clones were transferred to 5 ml of medium along with 0.2 ml of 0.25% solution of Chlorostrep (Chloromycetin and Streptomycin) to check bacterial growth. The amoebae grew well in this medium though their rate of multiplication varied from clone to clone as they were isolated from different categories. Six clones were initiated from ALA sample in the same way as was described for NDAC and AAD cases.

The present medium and method is well suited for initiating and maintaining clone cultures of *E. histolytica*. The advantage of the present media over Robinson's media used by Farri is that the same egg-slants can be used 3–4 times to pool the overlay for cloning. Moreover, clones isolated from different categories grew well irrespective of their origin. Gillin and Diamond[4] made colonies of *E. histolytica* on semisolid agar suspension. The amoebae grew well in the suspension-forming colonies. But to pick up a single colony from the tube having many colonies seems difficult. Das used axenic cultures of *E. histolytica* and other amoebae for cloning in perspex-cavity side. The clones grew well in cavities with TyI-S-33 medium but to know the exact biology of *E. histolytica* clones are required from indigenous strain.

In the present study, a success rate of 80–83% was obtained for acute cases and 60% for chronic cases. Farri reported a success rate of up to 80% with some strains when 48-h-old cu.tures were used. In the present study 24-h-old-cultures from acute cases and 72-h-old cultures from chronic cases did well for cloning.

The medium and methodology reported in this communication is recommended for initiating and maintaining clones of *E. histolytica* with a success rate of 80 to 83%.

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Effect of polyamine precursors and α-difluoromethylornithine on the course of *Trypanosoma evansi* infection in experimental albino mice

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The exogenous supply of arginine and ornithine to *Trypanosoma evansi*-infected mice prolonged mean survival time of the host although a three-fold increase in parasitaemia was noticed. Administration of the α-difluoromethylornithine, a specific suicide inhibitor of polyamine biosynthesis, reduced the parasitaemia and prolonged the survival time of the host. The possible implications of these results in relation to polyamine biosynthesis are discussed.

TRYPANOSOMIASIS is one among the six tropical diseases selected by the World Health Organization for its control and chemotherapy. The antigenic variation of trypanosomes is hampering the vaccine development against these parasites. Chemotherapy has, therefore, become important for the control of this disease[1]. There is an increasing emphasis on the exploitation of trypanosome metabolism for the rational screening of antimetabolites for chemotherapy, particularly with reference to growth-promoting metabolites[2]. Polyamines are considered to be important growth factors in prolife-rating cells and microorganisms[3]. It has been suggested that they play a vital role in trypanosome growth and metabolism[4]. Since arginine and ornithine are the important precursors in polyamine biosynthesis, an attempt is made to study the influence of these amino acids and α-difluoromethylornithine (DFMO) on *in vivo* growth of *Trypanosoma evansi*, an important pathogenic blood protozoan parasite of cattle in India.

T. evansi collected from the blood of infected cattle was maintained in laboratory animals through syringe passage. Experimental infection was initiated by the intraperitoneal (i.p.) injection of 10^5 parasites. The details about the methods of trypanosome separation from blood and inoculation are described elsewhere[5]. Inbred Swiss albino mice (30–35 g body weight) were used in the present study. Fifteen male mice were