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A preliminary study on amphizoic amoebae with special reference to their preference for bacterial food

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Abstract: The present study was planned to screen the growth pattern of six different species of pathogenic and non pathogenic amphizoic amoebae viz. *Naegleria fowleri, N. gruberi*, *Acanthamoeba culbertsoni*, *A. rhysodes, A. polyphaga* and *A. glebae* using six different bacterial species like *Escherichia coli (*Strain E_1 and E_2 and *E. coli lactose +ve*), *Proteus, Klebsiella* and *Pseudomonas* as food in their *in vitro* growth on non-nutrient agar medium. It was observed that out of six amoebae used; the pathogenic *N. fowleri* and *A. culbertsoni* were feeding on *E. coli* (all the strains). Feeding these two species of bacteria, the growth of these two amoebae was luxuriant, but not so good while feeding other strains of bacteria though they fed, survived and formed cysts. The remaining four amoebae were found to feed and survive only on *E. coli* (all the strain) and formed cysts but showed very poor growth while feeding on other four bacterial strains. It was inferred that *E. coli* is the most suitable bacterial species for *in vitro* growth of amphizoic amoebae for various purposes. This also reiterates that there exists a complex inter-relationship between amoebae and bacteria in different habitats.

Keywords: Amphizoic amoebae, Bacteria, Micro-ecosystem

INTRODUCTION

Amphizoic amoebae feed on a variety of bacteria, cyanobacteria (blue-green algae) and yeast (Rodriquez-Zarrogoza, 1997). But they are main predators of bacterial population and play a major role in the ecological balance of environmental system. Jjemba (2001) observed the interaction of protozoa with their potential prey bacteria in the rhizosphere and concluded that the differences in the colonization ability of test bacteria were not attributed to inherent differences in their susceptibility to predation by protozoa. It is well known that amoebae feed on living micro-organisms and that they ordinarily ingest several different kinds (Mast, 1939). It has however been demonstrated that for some species, one kind suffices for growth (Oehler, 1916, 1942; Rice, 1935 and Hopkins, 1937).

There exists a complex inter-relationship between amphizoic amoebae and bacteria in an aquatic system. The aim of present study was to discover weather amoebae may be affected by the quality of bacterial food or, conversely they are only affected by the proportion of available edible and non-edible bacterial species.

MATERIALS AND METHODS

For this study, six bacterial species such as *E. coli* (strain E_1 and E_2), *Proteus sp.*, *E. coli lactose* positive, *Klebsiella* and *Pseudomonas pyocyanea* (strain P_1) were selected. *E. coli* has already been reported to be the most preferred bacterial food (Singh, 1941). So *E. coli* (strain E_1) has been used as control. All the selected bacteria from 24 to 48 hours old culture, grown on nutrient agar slopes, were used as food for amoebae in the present study.

As amoebae generally grow and move in all the direction in the agar plates, when they are placed in the centre of bacterial circle. 15-20ml of sterilized non-nutrient agar (2.5-2.7% w/v of Hi-Media), pH 6.6-7.0 was poured in the pre-sterilized Petri dishes. After 1-2 hours when non-nutrients agar had solidified properly, the agar film was cut in six radii leaving a circle in the centre by a sterilized knife (Singh, 1941).

Young actively multiplying amoebae of a particular strain maintained at 37^0C on non-nutrient agar plates pre-seeded with *E. coli* were used as inoculum, which was placed in the centre of agar film radii. Different bacterial streaks were made in each agar radii touching the amoebae inoculum, and the plates were incubated at 37^0C for 8-10 days. For the comparative study of the food preference, bacteria of the same age and approximately in the same quantity were employed. After reaching a suitable bacterial food supply, the amoebae consumed bacteria, multiplied and moved along the bacterial streaks when they reached the non-edible bacterial food centre they either died or encysted without apparently consuming these bacteria. The preference for one type of food as opposed to the other is judged by the amount of bacteria consumed in a given time. This was observed even by

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Table1. *In vitro* selectivity of some amphizoic amoebae in preferring bacterial species as food.

S.No.	Amoebae	Strain	*E. coli* (E1)	*E. coli* (E2)	*Proteus*	*E. coli lactose +ve*	*Klebsiella*	*Pseudomonas*
1.	*N. fowleri*	RK-2	RC	RC	PC	NC	RC	NC
2.	*A. culbertsoni*	RK-1	RC	RC	SC	NC	RC	NC
3.	*A. rhysodes*	RP-1	RC	RC	SC	NC	PC	NC
4.	*A. polyphaga*	RP-2	RC	RC	SC	NC	PC	NC
5.	*N. gruberi*	GA-2	RC	RC	PC	NC	PC	NC
6.	*A. glebae*	MG-1	RC	RC	NC	NC	PC	NC

RC= Rapidly and completely consumed,SC= Slowly but completely consumed,C= Partly consumed,NC=Entirely non-consumed

naked eyes and at low magnification under microscope.

RESULTS AND DISCUSSION

This study was performed on bacterial food selectivity by using six pathogenic and non-pathogenic free-living amphizoic amoebae i.e. *Naegleria fowleri, N. gruberi, Acanthamoeba culbertsoni, A. rhysodes, A. polyphaga* and *A. glebae*. A total six bacterial species were also selected, to be used as food. Among two bacterial strains, each of *E. coli* (E_1 and E_2) and *Pseudomonas pyocyanae* (P_1) were considered and *E. coli* (E_1) was used as control. Other bacterial species used were *Proteus sp*., *Klebsiella sp*., *E. coli lactose +ve*. Growth and survival of amoebae in the presence of bacteria depends on the species and density of particular bacteria. Edible bacteria are ingested and digested inside the amoebic ell.

For the convenience of the present study, bacteria were classified on the basis of edibility by amphizoic amoebae into four categories Table-1

i) species rapidly and completely consumed (RC), ii) those species that were slowly but completely consumed (SC) iii) those species that were partly consumed (PC) iv) entirely non-edible species (NC)

In the present study all the test gram negative bacteria were found to be edible except the strain of *Pseudomonas sp.* and *E. coli lactose +ve*, which were found to be entirely non-edible species (NC). Similar results were also reported by Singh (1942 and 1945); Chang (1960); and Danso and Alexandra (1975).

Interaction between 'Limax' amoebae and bacteria results in alterations of the amoebae as well as of the bacteria (Walochnik *et al.,* 1999). Moreover the data of Larkin and Easty (1990) indicated that ingestion and metabolism of bacteria enhance virulence and pathogenicity of amoebae. Recently it was suggested that also molecular changes influencing the amoebic virulence e.g. in *Entamoeba histolytica* (Bhattacharya *et al.,* 1998).

Gram positive bacteria such as *E. coli lactose +ve* and *Pseudomonas* sp. were also found to be entirely non-edible species by all the species of amphizoic amoebae selected for the present study. Our results are inconformity with that of Chang (1960) and Rodriquez-Zarrogoza (1997). They had also reported that small free-living amphizoic amoebae grew well on agar along with variety of gram negative species of bacillary bacteria. Chang (1960) noted that when *Naegleria sp*. reaches non-edible bacteria, the amoebae either encysted or moved away. Marciano-Cabral (1988) observed that *N. fowleri* moved towards *P. aeraginosa*, but once in close proximity to the bacteria is not ingested and began to encyst.

According to Marciano-Cabral (2007) that the different bacterial species conforms that when the amoebae were near ingestible bacteria they move towards the bacteria by pseudopodia formation. *N. fowleri* appeared to respond to bacteria by three interrelated but distinct process (a) chemo kinesis (b) chemo taxis and (c) formation of food encystment of the amoebae even though prey cells were clearly visible within the food vacuole. Previous studies showed that protozoa digest the bacteria having the cell wall two to five times thicker than the cell walls of other gram-negative bacteria, a feature which influences digestibility (Gonzalez *et al.,* 1990 , Callieri *et al.,* 2002 and Pickup *et al.,* 2007).

This work on selectivity of bacterial food by amoebae revealed that the species of bacteria, such as *Klebsiella sp*. and *E. coli* were rapidly and completely consumed (RC) by all species of amoebae used in the present study. Similar results were also obtained by Singh (1945). Marciano-Cabral (1988) also reported that *Naegleria sp*. was often maintained in cultures containing bacteria such as *Klebsiella sp*. But it was observed that *E. coli* (E_1) was more preferred by *Naegleria gruberi, N. fowleri, Schizopyrenus russelli* than strain (E_2) (Weekers *et al.,* 1993).

The reasons for the apparent choice in bacterial food have not been so far clarified. The productions of certain types of pigments and bacterial toxins have been assumed responsible for their non-edibility (Singh, 1945), which provided protection to bacteria against invasion by amoebae. This is evident by rejection of *Pseudomonas pyocyanae* as bacterial food of amoebae in the present study also. With the observation of present study it is obvious that among the preferred bacterial species as food of amoebae, are mainly gram-negative bacteria. Gram-positive bacteria are entirely non-edible.

Amphizoic amoebae feed on various bacterial populations and thus, regulated bacterial number and

possibly enhance soil fertility (Singh, 1975). This inter relationship is of great ecological importance and can be used for the welfare of environment and mankind.

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