Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 95, No. 4, July 1986, pp. 469–473.

Growth of *Acanthamoeba culbertsoni* (Singh and Das 1970) and *Acanthamoeba rhysodes* (Singh 1952) (Protozoa: Gymnamoebia) in cultures

MRINAL GHOSH* and AMALESH CHOUDHURY
Department of Marine Science, University of Calcutta, 35, Ballygunge Circular Road, Calcutta 700 019, India
*Ecology Division, Zoological Survey of India, 8, Lindsay Street, Calcutta 700 087, India

MS received 2 January 1986

Abstract. Six strains of amoebae belonging to two species, viz *Acanthamoeba culbertsoni* and *Acanthamoeba rhysodes*, were isolated during the 3 prominent seasonal periods of premonsoon, monsoon and postmonsoon from the south of Sagar Island facing Bay of Bengal (21°56′ to 21°88′ N and 88°08′ to 88°16′E). The actual sampling sites were the lowest low tide belt areas. They were co-cultured in pairs, for respective seasonal periods, in 20‰ saline distilled water agar medium with their habitat associated microbiota as food. Dominance of all the seasonal isolates of *Acanthamoeba culbertsoni* over the corresponding strains of *Acanthamoeba rhysodes* arising out of competitive growth has been observed. Thus in laboratory maintained cultural conditions, strains of *Acanthamoeba culbertsoni* show remarkable adaptive superiority over those of *Acanthamoeba rhysodes*.

Keywords. *Acanthamoeba culbertsoni; Acanthamoeba rhysodes*; co-culture.

1. Introduction

Microhabitat, being the unit of protozoan ecology, may indeed be invaded by many species, but successful colonization depends on interaction with other organisms (Bamforth 1981). Stout (1952) pointed out that different species of soil protozoa occur in characteristic groupings which can be correlated with soil types. Protozoan communities are characterized by their short life history and rapid turnover which results in the availability of nutrients for recycling and the enhancement of the overall metabolic activity of the ecosystem (Stout 1974). Stout (1974) differentiated two basic patterns of organizations in most protozoan communities: one is dependent upon bacterial proliferation while the other is associated with some form of plant growth. It is now widely known that soil protozoan fauna exhibit a marked tolerance to varying environmental conditions with the ability to react quickly to favourable and unfavourable factors. Thus we see, in soils of high productivity there is the predominance of naked forms including flagellates, ciliates and naked amoebae. It is evident that rhizopods can exploit the large 'benthic' surface offered by shallow moisture films favouring interface locomotion (Bamforth 1980).

The amoeboid way of life is ecologically very successful because crawling forms can move in those shallow films; they can enter tiny spaces with their pseudopods; and they can change course merely by pushing out their body in a new direction (Bamforth 1981). Naked amoebae including 'limax' forms (e.g., *Naegleria, Vahlkampfia, Hartmannella*), *Acanthamoeba, Thecamoeba* and *Mayorella* are ubiquitous and usually furnish 50–90% of the protozoa in soils and litters (Bamforth 1980). Large amoebae including *Vampyrella, Biomyxa* and *Gephyramoeba* are also found. Additionally,

genera like *Naegleria* and *Tetramitus* can transform into flagellate stages, thus exploiting swimming locomotion.

This overall dominance amongst the protozoans in soil ecosystem is paralleled by their interspecific competition. Members of the genus *Acanthamoeba* have been found as common inhabitants of the coastal mangrove fringing belts of lower deltaic Bengal (Bhattacharya *et al* 1985; Choudhury and Ghosh 1985; Ghosh and Choudhury 1985; Ghosh *et al* 1985). A detailed investigation on their quantitative abundance in mangrove litter-soils revealed the dominance of *A. culbertsoni* over *A. rhysodes* and *A. astronyxis* (Ghosh *et al* 1985). It has already been reported that the lowest low tide isolates of *Acanthamoeba* can well be grown in saline distilled water agar (SDWA) medium (Ghosh and Choudhury 1985). In the present investigation seasonal isolates of *A. culbertsoni* and *A. rhysodes* from sand-silt substrata of the lowest low tide belt were co-cultured in 20‰ SDWA medium. Couple of strains isolated during the same seasonal period but members of one species or the other, were used at one particular experimental set. The overall growth then would reflect the relative dominance, if any, of one particular strain over the other, under such situations.

2. Materials and methods

2.1 *Sampling*

Samples from 3–5 cm depth of sand-silt beds of the lowest littoral zone were collected aseptically. These sites are normally exposed during the lowest ebb of spring tide for 2–3 days per fortnight while during neap tide remain submerged to depths of about 2–3 metres. All the samples were transported to the laboratory and processed within 24 hr.

2.2 *Isolation and identification of the strains*

Seasonal samples were inoculated on to food microorganisms in respective bay water agar petri dishes. Filtered bay water served as the fluid base. Clones of *A. culbertsoni* and *A. rhysodes* strains were established according to the method of Singh and Hanumaiah (1979). Identity of the species were confirmed by reacting the homogenates with anti *A. culbertsoni* and anti *A. rhysodes* sera raised in rabbit. These isolates were maintained through subcultures, once a month to once a quarter.

2.3 *Food source*

Since amoebae are capable of using a wide range of food sources, predominantly microflora, we used their habitat associated microbiota as food. These organisms from respective samples were grown on saline adjusted bacterial nutrient agar slopes. Ingredients required were: 1 litre of glass distilled water (pH 6·4); NaCl, 20·0 g; Bacto agar (Difco Laboratories, USA), 20·0 g; Na_2HPO_4, 2·60 g; glucose, 2·0 g and Bacto tryptone (Difco Laboratories, USA), 20·0 g. The mixture was boiled in water bath, dispensed suitably and autoclaved.

A little quantity of samples were inoculated aseptically on agar slopes and kept overnight at 37°C incubator. To avoid unwanted particles etc., they were subcultured

several times. These mixed cultures of food microbiota were used for isolation and subsequent maintenance of respective clonal isolates.

2.4 *The culture media*

2.4.1. *Isolating medium*: One litre of filtered seasonal bay water from the littoral zone and Bacto agar 15·0 g were boiled and autoclaved.

2.4.2. *Experimental medium*: One litre of glass distilled water; NaCl 20·0 g and Bacto agar 15·0 g pH was adjusted to 8·25 which is the approximate mean value of seasonal sampling water. These mixtures were boiled, autoclaved and dispensed in sterile 7·5 cm dia petri dishes. Media plates were suitably dried at room temperature before use.

2.5 *Modified Page's amoeba saline*

The modified Page's amoeba saline (PAS) (Page 1966) was made as follows: 1 litre of glass distilled water; NaCl, 20·0 g; $MgSO_4 \cdot 7H_2O$, 0·004 g; $CaCl_2 \cdot 2H_2O$, 0·004 g; Na_2HPO_4, 0·142 g and KH_2PO_4, 0·136 g.

2.6 *Experimental*

Laboratory maintained young cultures of microbiota were used as food. Final wet weight suspensions of 0·05 g/ml were made in 3 tubes for each of the 3 experimental sets for 3 seasonal isolates in modified PAS. Five replicate plates for each pair of competitive strains for 3 seasonal periods were spread with 0·2 ml of the suspending fluid so that each gets about 0·01 g wet weight of microorganisms. When the excess fluid was absorbed, cysts in pairs, for respective isolates were inoculated on media plates. The PAS medium was allowed to absorb and the plates were inverted and kept at 25 ± 1°C BOD incubator. Data for the overall population growth after 100 days of culture were taken and the values were expressed in counts/petri dish (mean of 3 replicate plates). Both the co-culturing strains as well as those growing separately on parallel cultures were considered. It is always probable to lose some of the organisms while harvesting.

3. Results

The growth of all the competitive sets of strains for all the 3 seasonal periods have been documented. The same of parallel cultures of seasonal isolates of *A. culbertsoni* and *A. rhysodes* have also been plotted. Overall growth of the experimental strains after 100 days of culture are plotted in figure 1. Maximum growth were encountered for monsoonal isolates in both the species being followed by postmonsoon and premonsoon growth in sequence. This bar diagram also depicts the dominance of all the strains of *A. culbertsoni* over the corresponding strains of *A. rhysodes*.

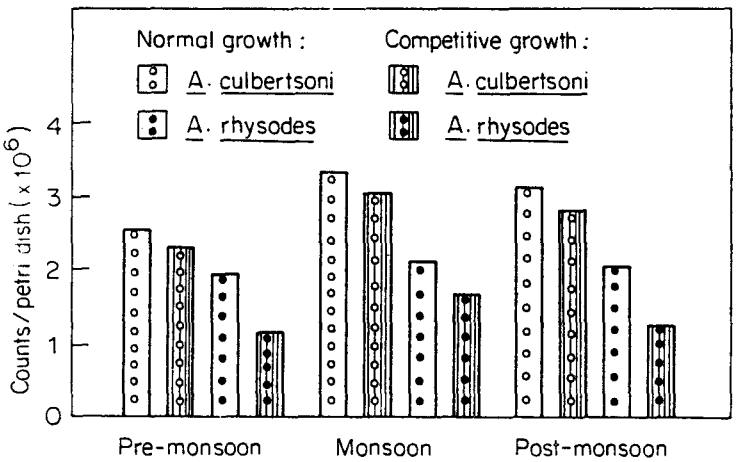

Figure 1. Growth of *A. culbertsoni* and *A. rhysodes* strains under co-cultural condition in 20‰ SDWA medium.

4. Discussion

Dominance is an expression of ecologic inequalities, arising out of different exploitations of the environment (McNaughton and Wolf 1970). Protozoa constitute a relatively small component in ecosystems with a large biomass, while in ecosystems with much smaller biomass they may constitute a much higher proportions. In ecosystems with fluctuating biomass they exhibit concomitant changes (Stout 1974). Competition can cause niche differentiation while small changes in the activity of microbial populations in soil can affect significant changes in soil metabolism (Stout 1973). The relative abundance of the 3 species of *Acanthamoeba*, viz *A. culbertsoni*, *A. rhysodes* and *A. astronyxis*, from mangrove litter-soils, both in the active and inactive stages has already been investigated (Ghosh *et al* 1985). In the present study, under cultural condition, the dominance of the strains of *A. culbertsoni* over those of *A. rhysodes* is clearly evident. This dominance however, might result from the presence of more amount of food amongst the supplied heterogeneous microbiota preferred by *A. culbertsoni* strains in relation to those of the competitive strains, or that they could have interacted with microbiota in a better way or else, they could inhibit the growth of *A. rhysodes* strains by producing some excretory and/or secretory factors in the culture medium while themselves being less affected by those produced by the competitive strains.

Acknowledgements

The senior author is grateful to Dr B K Tikader, Director, Zoological Survey of India, for laboratory facilities and the award of fellowship. Antisera were kind gifts from Dr S R Das, Microbiology Division, Central Drug Research Institute, Lucknow.

References

Bamforth S S 1980 Terrestrial Protozoa; *J. Protozool.* **27** 33–36

Bamforth S S 1981 Protist Biogeography; *J. Protozool.* **28** 2–9

Bhattacharya A, Ghosh M and Choudhury A 1985 Seasonal population dynamics of *Acanthamoeba rhysodes* (Singh 1952) in a mangrove litter-soil ecosystem of lower deltaic Bengal, India; *Abstr. VII Int. Cong. Protozool.*, Nairobi, Kenya

Choudhury A and Ghosh M 1985 Benthic gymnamoebae as micropredator components in a non-reclaimed mangrove swamp of Gangetic-estuary, India; *Abstr. VII Int. Cong. Protozool.*, Nairobi, Kenya

Ghosh M and Choudhury A 1985 Culturing gymnamoebae from saline environments of Hooghly estuary, India; *Abstr. II Natl. Seminar on Mar. Intertidal Ecol.*, Waltair

Ghosh M, Bhattacharya A and Choudhury A 1985 Competition amongst intertidal benthic *Acanthamoeba* spp. of Hooghly estuary, India; *Abstr. VII Int. Cong. Protozool.*, Nairobi, Kenya

McNaughton S J and Wolf L L 1970 Dominance and the niche in ecological systems; *Science* **167** 131–139

Page F C 1966 *Cryptodifflugia operculata* n. sp. (Rhizopodea: Arcellinida, Cryptodifflugiidae) and the status of the genus *Cryptodifflugia*; *Trans. Am. Microsc. Soc.* **85** 506–515

Singh B N 1952 Nuclear division in nine species of small free-living amoebae and its bearing on the classification of the order Amoebida; *Phil. Trans. R. Soc. London* **B236** 405–461

Singh B N and Das S R 1970 Studies on pathogenic and non-pathogenic small free-living amoebae and the bearing of nuclear division on the classification of the order Amoebida; *Phil. Trans. R. Soc. London* **B259** 435–476

Singh B N and Hanumaiah V 1979 studies on pathogenic and non-pathogenic amoebae and the bearing of nuclear division and locomotive form and behaviour on the classification of the order Amoebida; *Indian J. Microbiol.* (Monogr. No. 1 of the Assn. of Microbiol. of India) 1–80

Stout J D 1952 Protozoa and the soil; *Tuatara* **4** 103–107

Stout J D 1973 The relationship between protozoan populations and biological activity in soils; *Am. Zool.* **13** 193–201

Stout J D 1974 *Biology of Plant Litter Decomposition*, (eds) C H Dickinson and G J F Pugh, (New York: Academic Press) Vol. 2, pp 385–420