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Research Article

EFFECT OF PHYSICO-CHEMICAL FACTOR (pH) ON CYST FORMING SPECIES OF AEROBIC FREE LIVING AMPHIZOIC AMOEBAE

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ASTRACT
In the present study, the *in vitro* effect of different levels of pH i.e. 3, 4, 5, 6, 7, 8, 9 and 10 were observed on the entire cyst forming species of aerobic free living amphizoic amoebae isolated from different water samples. Observations were recorded instantly and after 8, 24, 48 and 72 hours of incubation at 25°C-30°C temperatures. Present study confirmed that pH alone was not effective on disease causing pathogenic species of Naegleria and Acanthamoeba, but association with other chemical like chlorine can be effective.

Keywords: *Amphizoic Amoebae, pH, Naegleria, Acanthamoeba*

INTRODUCTION
pH is an expression of hydrogen ion concentration in water. Specifically, pH is the negative logarithm of hydrogen ion (H^+) concentration (mol/L) in an aqueous solution. pH affects most chemical and biological processes in water, and it is one of the most important environmental factors limiting the distribution of species in aquatic habitats. Different species flourish within different ranges of pH, with the optima for most aquatic organisms falling between pH 6.5-8. U.S. EPA water quality criteria for pH in freshwater suggest a range of 6.5 to 9. Fluctuating pH or sustained pH outside this range reduces biological diversity in streams because it physiologically stresses many species and can result in decreased reproduction, decreased growth, disease, or death (Gashchin, and Viten'ko, 2011).
Protozoa are single cell organisms with in the Kingdom Protista which grow well in the humid environment. The important factor that effects the survival as well as species number of protozoa is food. Most of the protozoa feed on bacteria while some are capable of photosynthesis and other can absorb dissolved organic substances caused by bacterial decomposition. A side from food, almost all protozoa needs oxygen for their living. Hence, dissolved oxygen is an important factor effecting the growth of protozoa. The abundant areas for the survival and growth of protozoa are fresh water resources available with moderate amount of bacteria and dissolved organic substances as well as proper oxygen, while in the water with defined factors only specific type of protozoa would be found. Present knowledge of the survival effects of pH on amoebae is generally limited. Most studies that have been reported on *Paramecium* and pH largely concern the effect of pH on the forward swimming speed of the organism (Otter *et al.,* 2005). While the toxicity effect of extreme values of pH on some species of *Paramecium* including amoeba proteus has been reported (Rao *et al.,* 2006), few studies under laboratory conditions performed to simulate those hydrogen ions that usually occur in the natural habitat. The pH in natural, inland waters varies from 4.0 to 9.0 (Wetzel, 2001).
Small free living aerobic amoebae belonging to the genera *Naegleria*, *Acanthamoeba* and *Balamuthia* (Zsuzsanna *et al.,* 1998; Jain *et al.,* 2002; Kaushal *et al.,* 2008; Jeong and Yu, 2005) that could be known to cause diseases in human beings and animals. These amoebae were regarded as amphizoic opportunistically pathogenic in nature, producing fatal human diseases affecting central nervous system and eye (Butt, 1966; John, 1993; Marciano-Cabral and Cabral, 2003). Amphizoic amoebae (pathogenic stains of *Naegleria* and *Acanthamoeba*) are mainly responsible for infections in brain and eyes and causing primary Amoebic Meningoencephalitis (PAM) Granulomatus Amoebic Encephalitis (GAE) and

Amoebic Keratitis (A.K) in human beings and domestic animals, (Morales *et al.*, 2006; Daft *et al.*, 2005). These amoebae normally do not cause disease, but live as phagotroph in ponds, lakes, rivers, streams, soil etc. where they feed on bacteria. However, as opportunist, they infect Central nervous system and eyes. Actually, Page (1974) used the term Amphizoic for these amoebae, because they have the ability to live both as free living as well as endoparasites. *N. fowleri* is the causative organism of acute infection of healthy persons having recent history of swimming and the disease is termed as Primary Amoebic Meningocephalitis (PAM) whereas, *A. culbertsoni* in the etiological agent of chronic illness of immunological weak individuals having no history of swimming and disease is termed as Granulomatus Amoebic Encephalitis (GAE). *A.polyhaga* and *A.castellanii* are known to cause eye infections and the disease caused by them is referred as Amoebic Keratitis (Culbertson, 1971).

This study was therefore, undertaken to examine the laboratory survival effect of pH on free living amphizoic amoebae and tolerance of these amoebae under normoxic conditions. In this study normoxic condition was defined as air-aerated for the media.

MATERIALS AND METHODS

Amoebae were isolated from various water sources from the Lucknow city. Following method (Singh, 1955) was utilized for the isolation of small free living aerobic amoebae from various water samples, about 2 liters of river or pond water was collected in a sterilized jar. This water in the laboratory was filtered though a sterilized filter paper. The cone of filter paper was cut by 1cm with the help of sterilized scissors. This small cone was placed at the center of the Petri dish already seeded with *E.coli*. The plates were incubated at 37°C for 8 to 10 days for the growth of amoebae. The plates were examined daily for the cystic and trophic structures under low power of microscopes. For the proper biological characterization of amoebae, their morphology, locomotion, nuclear division and other characters will be studied following the standardized methods of Singh and Hanumaiah (1979). To determine the *in vitro* effect of pH on free living amphizoic amoebae, following method was used. Large no. of young and actively multiplying amoebae of same strain from a clonal culture were maintained on non-nutrient agar plates seeded with E. coli as food. They were harvested in phosphate buffer saline (PBS) and then washed with distilled water 5 to 6 times by low speed centrifugation (400 rpm upto10 min) to remove bacteria as much as possible. After every washing, a uniform suspension of amoebae was made by shaking it. The amoebae were finally suspended in sterile distilled water (2x105cells/ml) and 0.05ml of the cell suspension containing 10,000 amoebae were placed in a glass cavity slides and the slides were put in a moist chamber. After 15-30 min, when the amoebae were completely stretched out and adhering to the glass surface, 10µl of pH solution of different levels (3, 4, 5, 6,7,8,9 and 10) was added to the cavity of the slide. Observations were recorded instantly after 8, 24, 48 and 72 hours of incubation at 25°C-30°C temperatures

RESULTS AND DISCUSSION

The natural water possesses various salts like sodium potassium, calcium, magnesium, chloride, sulphate, carbonate, bicarbonate; nitrate etc. which comes from washing of home/kitchen, agricultural field during rain fall and different industrial factories. These salts and ion are directly responsible for the salinity of water. The difference in the salinity of water acts as an important limiting factor for the distribution of different species of small free living amphizoic amoebae in water. Different species of amphizoic amoebae show affinity towards specific pH conditions. pH, a measure of hydrogen ion activity, is to express the intensity of acidic or alkaline condition of a solution. It is also an important factor in water analysis since it enters into the calculation of acidity, alkalinity and process like coagulation, disinfection and corrosion control.

Desirable pH range for drinking water is 7.0-8.5. Knowledge of pH is essential in the selection of coagulation for water purification for instance; aluminium sulphate is effective at pH 6.7 while ferrous sulphate coagulate well at a higher pH. *In vitro* effects of different levels of pH i.e. 3, 4, 5, 6, 7, 8, 9 and 10 were observed on the entire cyst forming species of aerobic free living amphizoic amoebae isolated from different water samples. Observations were recorded instantly and after 8, 24 and 48 hours of incubation at 25°C-30°C temperatures.

When these amoebae were exposed to pH 3 again instant reaction took place and all the trophozoites were transformed into cystic forms. But after 8 hours of expose small clumps started floating on the surface in the cavity slides. Their concentration were more in amoebae in amoebae like *Hartmanella* asp, *Schizopyrenus* sp. of amoeba like *Naegleria* sp. and *Acanthamoeba* sp. had comparatively less concentration of clumps. After 24 and 48 hours, no noticeable changes were observed.

When these amoebae were exposed to pH 4 again instant reaction took place and all the trophozoites were transformed immediately into cystic forms. But after 10 hours of exposure small clumps floating on the surface in the cavity slides. Less concentration of clumps were found on the *Naegleria fowleri* and *Acanthamoeba culbertsoni*.

After 24/48 and 72 hours of exposure no noticeable changes were observed. the result were the same as those observed by Carter (1970) who showed that free living *Naegleria fowleri* could survive in pH 3.45-8.50. The amoebae can proliferate in man-made ponds where the pH is 7.4-1.3 (De Jonckheere, 1977) since the pathogenic amoebae can proliferate in manmade ponds they may cause infection those who swim in the contaminated water.

When these amoebae were exposed to pH 5 there pseudopodia contracted instantly within a speck of time and trophozoites changed into rounded condition, here also the contractile vacuole was clearly visible. After 10 hours of exposure they transformed to cystic forms.

When these, pathogenic amphizoic amoebae were exposed to pH 6, their pseudopodia contacted gradually and trophozoites changed to rounded condition but contractile vacuole were clearly visible. After 10 hours of exposure they transformed into cystic forms and got stuck to the glass surface.

Trophic forms of the species of pathogenic amphizoic amoebae did not exhibit physiological change at pH 7 (neutral).

When amoebae were exposed to pH 8, it was observed of amoebae like, A. culbertsoni, exhibited the effects instantly but it was only in about 70% of all the experimental trophozoites, while remaining became sluggish, but still wee in there trophic forms.

At an exposure of pH 9 all the trophozoites instantly changed into cystic forms and after 10 hours of exposure, small clumps, of cyst could be seen. Our results show conformity with workers like Pandey and Sharma (2004), Paul and Sharma (2009), Shin and Im (2004).

At an exposure of pH 10, there was no noticeable change in the effect of the species on which the experiment was performed. All the trophozoites of every species transformed into cystic forms instantly and after 8 hours of exposure small clumps of cysts could be seen. Weik and John (1977) reported that growth on N. fowleri is accompanied by a pH increase in the medium.

But Sykora *et al.*, (1983) and Parija and Jayakeerhee (1999) stated the absence of strict dependency and ineffectiveness of pH in predicting the survival and virulence of N. fowleri at a range of 2.1-8.15. Acanthamoeba are better able to tolerate a range of growth conditions than Naegleria species. They readily survive over a wide range of osmolarities, both in vitro and in vivo, having been isolated from marine and fresh water; from tissue culture media, where they occur as contaminants and from soil (Schuster, 2002). This observation was in accordance to findings of the present study.

The pH range tolerated by free living amoebae is equally impressive. Present study confirmed that ph alone was not effective on disease causing pathogenic species of Naegleria and Acanthamoeba, but association with other chemical like chlorine etc. can be effective.

Since the pathogenic amoebae can proliferate at different level of pH showing alarming sign for disease causing infection in human being or animals that swim in the contaminated water and uses such water for daily purpose.

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