Survival of *Escherichia coli* in a tropical estuary

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ABSTRACT

The survival of Escherichia coli in tropical estuarine water has been studied under controlled laboratory conditions using microcosms. The survival has been assessed in terms of various self purifying factors of the natural waters such as biological, chemical and physical factors. The biological factors considered included competition from other microorganisms, predation by protozoa and coliphages. The suitability of the chemical composition of estuarine water has been studied under chemical factors and negative impact of sunlight has been studied under physical factors. The results revealed that sunlight exerted maximum negative impact, followed by biotic factors contained in the estuarine water. However, the chemical composition of the estuarine water is found to be suitable for the growth and survival of E. coli. The injury exerted by each of the above factors was also evaluated by using a selective and non-selective medium in conjunction. It was found that sunlight resulted in 100% injury of the cells as the cells failed to develop in a selective medium. While, sunlight resulted in the extinction of 90% of the E. coli cells within the first two hours of exposure, biotic factors took nearly 24 hours to remove the same amount of population.

Keywords*: Escherichia coli, estuarine waters, coliphages, protozoa, survival rate*

1 INTRODUCTION

One of the characteristic features of the estuarine system is the constant pollution from various human and non-human sources. Population explosion and rapid industrialisation has resulted in an ever-increasing load of waste input into this ecosystem. Due to this reason estuaries are generally referred to as the septic tank of the big cities. Large numbers of pathogenic bacteria enters this system mainly through sewage input. Rivers are the main contributors to the estuary, which transport a large volume of terrigenous materials and dump it in the estuary. However, all the natural systems have considerable self- purifying capacities owing to various physicochemical and biological parameters.

Most sanitary indicator organisms as well as the enteric water borne pathogens are bacteria whose natural environment is the intestine of man and warm-blooded animals. When discharged in the faeces, these microorganisms frequently gain entry into a body of water. Once these bacteria are deposited into the water, they are in an environment that is not favourable to the maintenance of viability of most bacteria. The survival of enteric bacteria in natural aquatic ecosystems has been of interest to public health and microbial ecology (Barcina *et al.* 1986; Borrego and Figueras, 1997; Catalao Dionisio *et al.* 2000).

Several factors are involved in the disappearance of the pollutant microorganism in the aquatic environment, the two most important being physical dilution and microbial inactivation. Both processes depend on various physicochemical and biological factors such as water temperature (Anderson *et al*. 1983), adsorption and sedimentation processes (Mitchell and Chamberlain, 1975), sunlight action, predation by bacteria or protozoa, bacteriophage lysis, lack of nutrients, competition with autochthonous microflora and antibiosis (Rhodes and Kator, 1990; Ricca and Cooney, 1999). However, there is considerable disagreement among the observations made by various researchers.

Escherichia coli is considered as typical faecal indicator bacteria and their presence in natural waters is considered as indicator for the presence of possible pathogens. However, their absence does not necessarily guarantee the quality of water (Dutka, 1973). Therefore, it is interesting to know the inactivation kinetics that environmental factors exert on this faecal indicator bacterium, since their survival rates in the aquatic environment may determine their ability as suitable indicator.

In the present investigation microcosm studies were carried out to determine the effects of various self purifying factors such as biotic, physical and chemical factors on the survival of *E. coli* in estuarine water.

2 MATERIALS AND METHODS

Test organism: *E. coli* isolated from the Cochin estuary was used.

Preparation of inocula: *E. coli*, cells were inoculated into Tryptone Soya Broth (TSB) and incubated overnight (16-18 hours) at 37°C. After incubation the cells were concentrated by centrifugation at 3000 rpm for 15 minutes and washed twice with sterile isotonic saline solution. After the final wash the cells were suspended in the same isotonic saline solution at a concentration of 10^8 colony-forming units per ml. From this final suspension 1 ml was inoculated into 250 ml Erlenmeyer flasks with 100 ml of the test solution so as to give an initial inoculum density of 10^6 cells per ml of test solution.

Test solution to study the effect of biological factors: Raw estuarine water with all its self-contained biotic factors was used. Estuarine water from different stations were collected and pooled and then a sub sample of 100ml was taken to suspend the test organisms.

The competing microflora in the estuarine water has been estimated by standard plate count method using nutrient agar prepared in filter sterilised estuarine water. Protozoans were analysed qualitatively with the help of a microscope. Bacteriophages were enumerated by plaque assay using the double layer agar method.

Test solutions to study the effect of chemical composition of the estuarine water: The negative impact of the chemical composition of estuarine water has been studied by suspending the test organisms in filter sterilised (0.22 micron) estuarine water, which excluded all the biotic factors, while preserving the dissolved organic components.

Test solution to study the effect of sunlight: Filter sterilised (0.22 microns) estuarine water was used. Test solutions were taken in sterile glass bottles, which were suspended at about half a fooot below the water surface in a glass tank (200 litre capacity) maintained at the roof top. The experiment started at 8 am and continued up to 6 pm with sampling at 2-hour intervals.

One set of all the test solutions were incubated at 30°C and the other at 20°C except the one supplemented with sunlight, which was incubated only at 30°C.

Enumeration techniques: The non-selective medium, tryptic soy agar (TSA) was used to recover the *E. coli* cells from the test solutions with spread plating technique and incubation at 37°C for 24 hours. The selective medium, eosine methylene blue (EMB) agar was used to evaluate the injury rate exerted by the different test solutions. The samples from the various test solutions were taken and assayed after 0, 1, 2, 3 and 4 days with the spread plating technique. All the samples were replicated two-fold. The percentage of survivors and injured cells at time 't' were calculated according the formula:

$$\text{Percentage of survival of } \textit{E. coli} \text{ cells at time 't'} = \frac{\text{Count on TSA plates at time 't'}}{\text{Count on TSA plates at time '0'}} \times 100$$

$$\text{Percentage of injury of } \textit{E.coli} \text{ cells at time 't'} = 1 - \frac{\text{Count on EMB plates at time 't'}}{\text{Count on TSA plates at time 't'}} \times 100$$

E. coli cells suspended in 100 ml isotonic saline solution were used as control.

3 RESULTS

The percentage of survival and injury of *E. coli* as a function of biotic factors (raw estuarine water) is given in table 1. The *E. coli* cells showed considerable reduction in the test solution. The T_{90} value i.e., time required to reduce 90 percent of the population, is reached within a day in the test solution kept at room temperature. In the test solution maintained at 20°C, T_{90} values were obtained after 26 hrs. The *E. coli* cells showed steady and rapid decline till the 3rd day and there after they showed signs of acclimatisation and recovery as evidenced by the increased population counts towards the end of the experimental period.

Table 1 Percentage of survival and injury of *E. coli* in raw estuarine water.

Test solution	Percentage of survival[a]				Percentage of injury[b]			
	Time (Days)				Time (Days)			
	1	2	3	4	1	2	3	4
Isotonic saline (control)	27.5	64.47	15.29	15.29	78.26	3.28	30	38.38
Raw water at 30°C	10.94	0.59	1.11	3.64	26.88	80	89.92	73.22
Raw water at 20°C	35.29	0.588	0.694	3.88	35.29	0.59	0.69	3.88

Table 2 represents the survival and injury percentages of *E. coli* in the filter sterilized estuarine water, i.e., as a function of chemical composition of the test solution. There was no negative impact of the test solution on the *E. coli.* The cells in fact showed considerable increase as the experiment progressed. The growth was slightly less at 20°C, which is possible, as the test organism is a mesophilic one. The injury levels were also less as indicated by the similar levels of counts on both selective and non-selective media.

Table 2 Percentage survival and injury of *E. coli* in filter sterilised estuarine water.

Test solution	Percentage survival				Percentage injury			
	Time (Days)				Time (Days)			
	1	2	3	4	1	2	3	4
Isotonic saline (control)	2.1	89.36	51.06	59.57	0	26	29	42.85
Filtered water at 30°C	146	159	170	159	0	5.33	16.25	18.66
Filtered water at 20°C	74.96	121	97.87	123	0	7	0	8.62

The sunlight had the most intense inactivation as evidenced by steep decline of cells in the duration of 4 hours (Table 3). The T_{90} values were obtained after 4 hours exposure to sunlight. The population declined steadily and did not show any signs of acclimatisation during the experiment duration of 8 hours. Injury levels were also very high throughout the experimental period. The extinction curves of *E. coli* cells suspended in raw estuarine water, i.e., as a function of biological factors, are represented in figure 1. The cells reduced by one log in 24 hours in the test solution maintained at room temperature (30°C). The cells maintained in the test solution at 20°C did not show any decline in the first 24 hours and then continue to decline very rapidly till the end of the experiment, the cells at room temperature show acclimatization and slow growth since the 2nd day. The control solution did not exert any considerable inactivation throughout the experiment. Figure 2 represents the extinction curves of *E. coli* in filter-sterilized water, i.e., as a function of chemical composition of estuarine water. The test solution maintained at 30°C did not show any considerable reduction in the number of cells but it showed slight growth since the 1st day and after it maintained a steady growth.

Table 3 Percentage survival and injury of *E.coli* in filter sterilized estuarine water supplemented with sunlight

Test solution	Percentage survival				Percentage injury			
	Time (Hours)				Time (Hours)			
	2	4	6	8	2	4	6	8
Filter sterilised water + sunlight	17.07	1.46	0.168	0.143	90	99.83	98.55	99.9

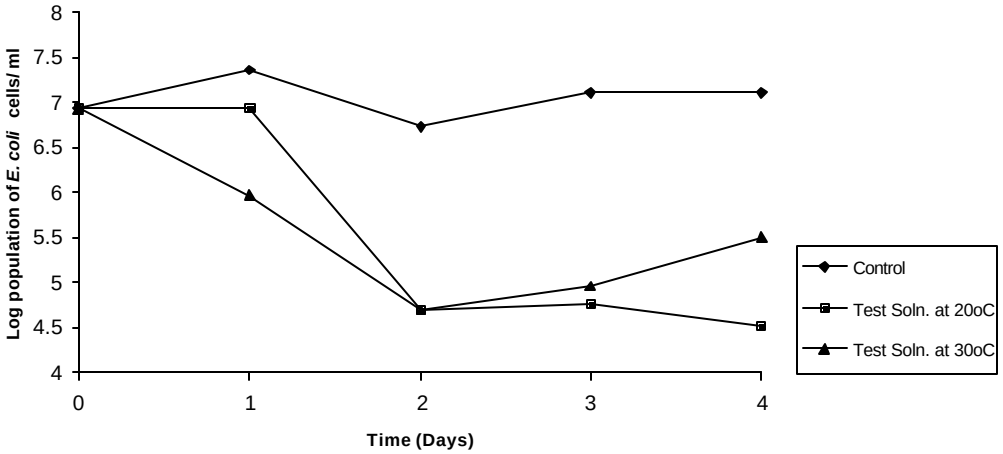

Figure 1 Suvival curves of *Escherichia coli* in estuarine water as a function of biotic factors

While the cells maintained at 20°C did not show any considerable decline of the cells. It showed acclimatization and maintained a more or less steady growth till the end of the experiment.

Figure 3 represents the extinction curve of *E. coli* in filter sterilized estuarine water supplemented with sunlight, as a function of sunlight action. The cells showed a sharp and continuous decline throughout the experiment. The cells showed 1 log reduction after 3 hrs exposure. The sunlight exerted very high inactivation effect on *E. coli* cells in the test solution.

4 DISCUSSION

In the present investigation, microcosm studies have been carried out to find out the survival of *Escherichia coli* in a tropical estuary. Results indicated a rapid inactivation of the suspended test organisms in raw estuarine water. The findings are in agreement with the observations of Morinigo *et al.* (1989) and Cornax *et al.* (1990), who studied the survival of indicator and pathogenic bacteria along the coast of Spain. The role of biological factors were further strengthened by our observations that when the biological factors contained in the estuarine water were removed by filtration (0.22 microns) the test organisms showed enhanced survival. Morinigo *et al.* (1990) also observed an extended survival of *Salmonella* and other indicator microorganisms in his studies using a membrane diffusion chamber, which prevents the entry of bacterial predators inside.

We have also estimated the total heterotrophic bacterial (THB) population contained in the estuarine water, which showed around 10^{5-6} cells per ml of the raw estuarine water indicating severe competition from these autochthonous microorganisms. Rhodes and Kator (1990) reported a higher mortality of *Escherichia coli* cells in the estuarine environment due to autochthonous microbiota. The possible predators such as protozoans and coliphages have also been assessed in the present investigation. Majority of the protozoans were found to be ciliates, which are reported to do active grazing on bacteria. Mitchell and Morris (1969) demonstrated the existence of microbial predators by adding untreated seawater to agar containing dense suspensions of *E. coli*, and observed discrete clear areas (plaques). Inspection of different plaques revealed a variety of protozoa and bacteria having lytic activity towards *E. coli*. Enzinger and Cooper (1976) reported that the survival of *E. coli* in natural waters is a function of protozoan predators and observed a higher number of protozoan predators resulted in a rapid decline of *E. coli* cells. Bacteriophages have also been considered as a factor in the removal of coliforms from natural environments. We were also able to detect the coliphages in the sample by plaque assay and the population varied around 1000 plaque forming units.

The suitability of the chemical composition of the estuarine water for the survival of *E. coli* has been assessed, by filtering out the biological factors from it as well as incubating in the dark in order to avoid interference from the light factor. The results indicated that the chemical composition of the estuarine water is well suited for the survival *E. coli*, which showed a gradual and steady increase in the number of cells throughout the experimental period. The cells showed a reduced growth at 20°C suggesting the mesophilic nature of this organism. The growth of the cells in the test solution may be due to the high level of nutrients

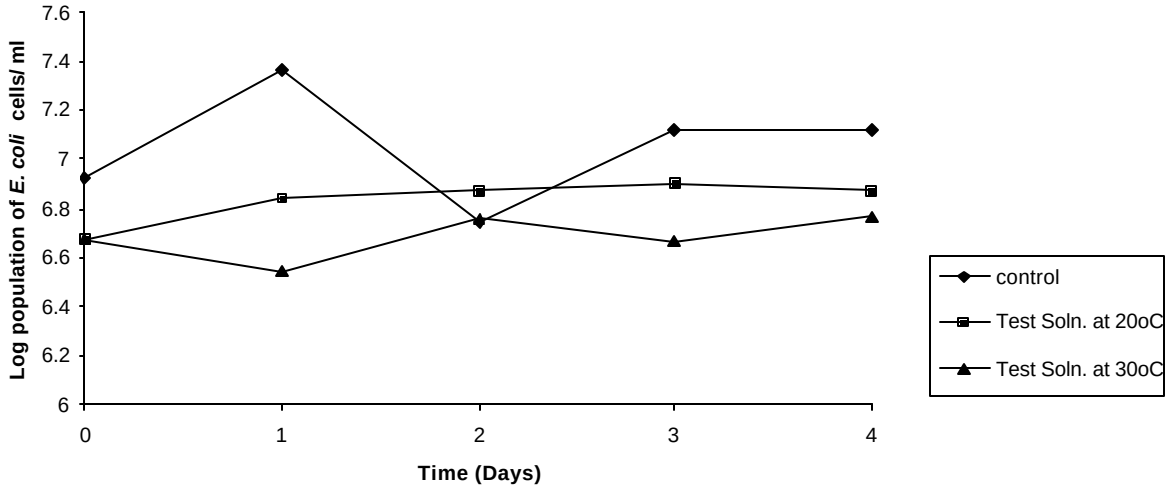

Figure 2 Survival curves of *Escherichia coli* in estuarine water as a function of its chemical composition

that are available in the estuarine water. The growth pattern also shows a utilization of the available nutrients in the initial days and then stagnation, possibly due to nutrient limitation.

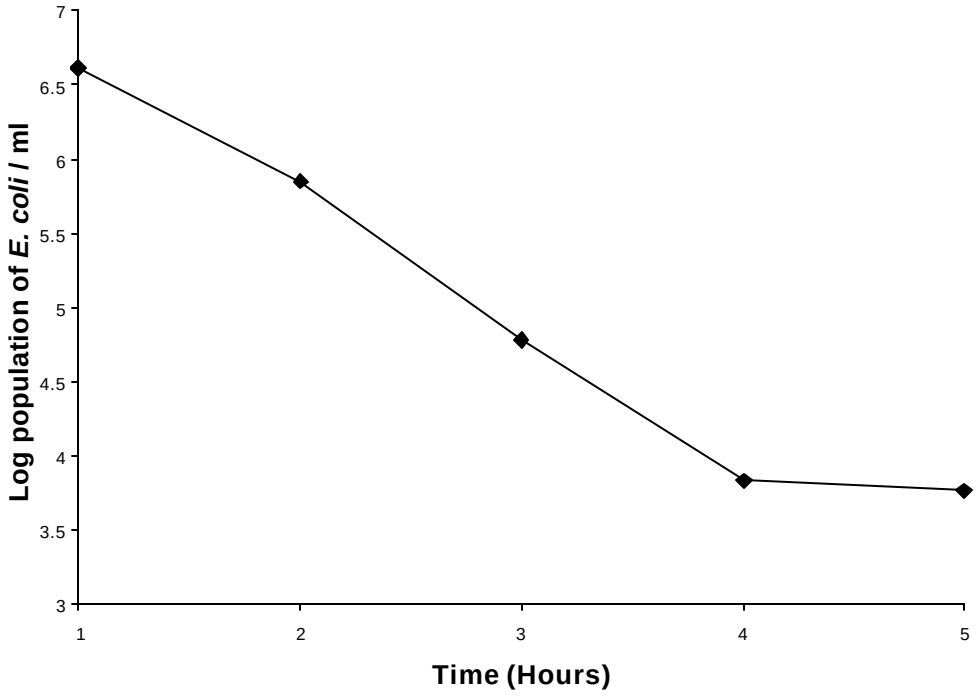

Figure 3 Survival curve of *Escherichia coli* in estuarine water as a function of sunlight.

Effect of sunlight on *E. coli* cells has been studied by suspending the test organisms in filter-sterilized water and exposing them to natural sunlight. The experiment has been conducted during the daytime for an 8-hour duration from 10 am to 6 pm. The results indicated remarkable inactivation of *E. coli*. The reduction of cells was linear in relation to time and the T_{90} values reached within 120 minutes. The observations agree with the findings of Fujioka *et al.* (1981) and Fujioka and Narikawa (1982) who reported sunlight as the major inactivation factor affecting the survival of indicator bacteria in the natural environment. The *E. coli* cells were found to acclimatize after 6 hours of exposure. The stabilization might be resulting from the recovery of the damaged cells or selection of more resistant organisms. Our findings are also in agreement with the observations of Sieracki and Sieburth (1986) and Rhodes and Kator (1990) who observed a higher mortality and sublethal stress during the first four hours of the experiment in their studies with *E. coli* in estuarine environment.

We had observed that the injury caused by the sunlight was almost 100% as the cells failed to develop on the selective medium. Bacterial injury describes a temporary state in which an organism is unable to reproduce during adverse condition, but still maintain some metabolic activity. The extent of sub-lethal injury produced in the stressed pathogenic population is directly related to the exposure time in the adverse environment. The injury level was much higher when compared to the injury of cells in the other test solutions such as raw estuarine water and filter sterilized estuarine water, which were incubted in the dark. The effect of the visible light may be the result of the accumulation of exogenous and endogenous peroxidases produced by the respiratory chain or catalase system (Kapuscinski and Mitchell, 1981).

The results of the present investigation revealed that sunlight is the most important inactivating factor on the survival of indicator bacteria such as E. coli in the estuarine water. While biological factors contained in the estuarine water such as protozoans and coliphages were also exerted considerable inactivation of these organisms, the chemical composition of the estuarine water did not exert any negative impact on the E. coli cells.

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