Fishery Technology
2000, Vol. 37(2) pp : 146 - 149

NOTE

Occurrence of Coliphages in Fish and Aquaculture Farms

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Coliphages were detected in water samples collected from brackish water and fresh water fish farms. Coliphages were also detected in the farmed fresh water fish, common carp and marine fish, oil sardine, from local market. Coliphage levels obtained were as follows:- water from brackish water fish farm 3 pfu.ml^{-1}, water from fresh water fish farm 23 pfu.ml^{-1}, fresh water fish 240 pfu.g^{-1} and marine fish 3500 pfu.g^{-1}.

Key words: Coliphages; *E. coli*, water quality

Bacteriophages or phages are viruses of prokaryotes including eubacteria and archaebacteria. Phages occur in many different habitats but they have essentially the same habitat as their host bacteria.

Water, regarded as hygienically safe because conventional bacterial indicators of faecal pollution such as coliform organisms and faecal streptococci could not be detected, may still contain infectious viruses. Epidemiological and microbiological findings have challenged the reliability of coliform bacteria as an indicator of the presence of enteric viral pathogen (Stetler, 1984; Calici *et al.*, 1998). Hence, there is a need for better model organisms to monitor water quality.

Payment & Farco (1993) advocated the use of bacterial viruses, especially, those infecting *Escherichia coli* (coliphages) for assessing the virological quality of drinking water. The survival of coliphages have been shown to be parallel to that of human enteric viruses (Havelaar & Hogeboom, 1984).

Aquaculture is all set to play a significant role in the food security and nutritional security of the country. Faecal wastes from animals, including humans, reach the aquatic environment as a result of run-off from the land during rainfall and/or emptying of sewage/drainage pipes. These aquatic bodies are the major source of water for fresh and/or brackish water aquaculture. Hence there is urgent need to monitor the aquaculture system so as to ensure public health.

The present investigation was done with an objective to detect coliphages in fish and pond water samples.

Coliphages were assayed by the double agar overlay method described by Kennedy *et al.*, (1986) with slight modifications. The assay was done by mixing 2 ml portion of sample suspensions with 1 ml of *E. coli* (6h culture in EC broth incubated at 44.5°C) and 3ml of molten (45°C) overlay EC agar (1% agar). This mixture was overlaid on pre-poured plates of EC medium (2% agar). The top agar layer was allowed to harden. The plates were then inverted and incubated at 37°C. Plaques were counted after overnight incubation. Plaques appear as clear zones (holes) on *E. coli* lawn. Coliphage count was expressed as number of plaque forming units (pfu) per gram or ml.

E. coli strains were screened for their ability to express maximum number of phages. For this, 9 *E. coli* strains viz., E1 and E2 from the culture collection of our laboratory; ET, isolated from water of brackish water prawn farm; WY, isolated from brackish water fish farm; EL2 and EL3, isolated from brackish water fish and L5, L10 and TY, isolated from marine fish were screened. Ten microlitres of four enriched phage suspensions were spotted on lawns of different *E. coli* strains. The strains E1 and ET expressed phage from all the four phage suspensions. Other strains could express only few. Dhillon & Dhillon (1974) found that wild strains from clinical materials were susceptible to a small number of phages in sewage. *E. coli* strain E1 expressed comparatively higher number of plaque forming units in water samples, by the double agar overlay method. Hence, E1 was selected as host strain for the assay of coliphages in water and fish samples.

Water samples collected from a brackish water fish farm (Kerala Agricultural University Fishery Station, Puduvypu) and a fresh water fish farm (Private Fish Farm, Thiruvankulam) were assayed for coliphages. The sample from brackish water fish farm had a coliphage count of 3 pfu.ml^{-1} whereas water from fresh water fish farm had a coliphage count of 23 pfu.ml^{-1}. The plaques in both the cases were uniform in shape and size. They were circular with a diameter of 3-4mm (Fig. 1).

The water samples were analysed for faecal indicator bacteria. The results are shown in Table 1.

Table 1. Faecal indicators in water samples from fish farms

Faecal indicator	Brackish water fish farm	Fresh water fish farm
MPN Faecal coliforms/100 ml	1400+	70
MPN *E. coli*/100 ml	300	30
Faecal Streptococci cfu/ml	10	7
MPN Sulphite Reducing Clostridia/100 ml	160	not tested

Composite samples of a fresh water fish (common carp from Fish farm, Thiruvankulam) and marine fish (oil sardine from local market) were assayed for coliphages. The coliphage count was 240 pfu.g^{-1} in carp sample whereas oil sardine had a coliphage count of 3500 pfu.g^{-1} There was difference in the morphology of plaques obtained from the two samples. The plaques from carp were relatively large (>4mm) but the size was not uniform. The shape of the plaques was roughly circular (Fig. 2). The plaques from oil sardine were uniform in shape and size (Fig. 3).The MPN faecal coliforms and MPN *E. coli* were over 140.g^{-1} in both the samples. Kfir *et al.* (1991)

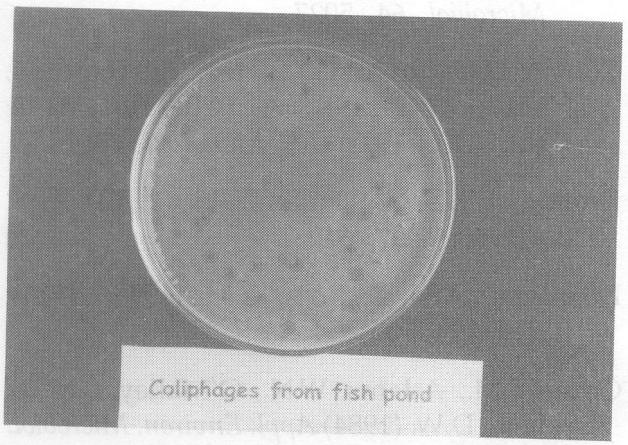

Fig. 1. Coliphages from fish pond.

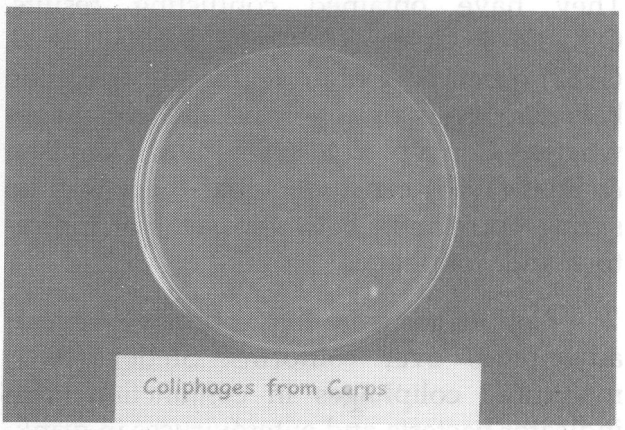

Fig. 2. Coliphages from carp.

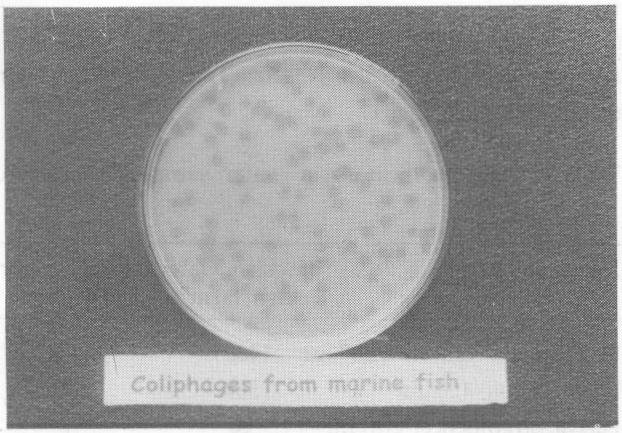

Fig. 3. Coliphages from marine fish.

observed that coliphages produce larger and clearer plaques than other bacteriophages. Coliphages were detected in mussel (Beril *et al.*, 1996) beef, chicken, pork and mixed vegetable samples (Kennedy *et al.*, 1986).

Coliphages were detected in all the samples where faecal coliforms and *E. coli* were detected. Coliphages appear to be more in fresh water than in brackish water whereas faecal coliforms and *E. coli* were more in brackish water than in fresh water. In the fish samples, coliphages, faecal coliforms and *E. coli* were all high. Coliphages were more in marine fish than in fresh water fish. Several researchers have tried to find out whether there was any correlation between coliphages and indicator bacteria. They have obtained conflicting results. O'Keefe & Green (1989) and Randall *et al.* (1982) observed a high degree of correlation between levels of coliforms and coliphages whereas Contato *et al.* (1995) and Donnison & Ross (1995) concluded that there was no strong correlation between bacterial indicators and coliphages.

Coliphages were found to have several advantages over coliforms. Stetler (1984) monitored coliphages in conjunction with indicator bacteria and enteroviruses in drinking water and concluded that enterovirus isolates were better correlated with coliphages than with total coliforms, faecal coliforms and faecal streptococci. The survival of coliphages have been shown to be parallel to that of human enteric viruses (Havelaar & Hogeboom, 1984). Power & Collins (1989) found that *E. coli* was eliminated more rapidly than polio virus and coliphage from shell fish during depuration. Coliphages were isolated from samples in which coliforms were undetectable (Amundson *et al.*, 1988).

Further studies are needed to compare the selected strain E1 with known *E. coli* phage host strains obtained from type collection centres. Thereafter, a large number of water and fish samples are to be analysed so as to assess the value of coliphages as indicators of quality of aquaculture produce, by comparing with commonly employed faecal indicator bacteria.

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