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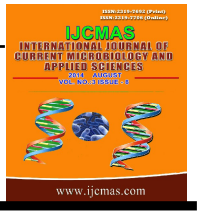

Short Communications

Isolation of *Listeria*- specific bacteriophage from three different towns in Kerala, India

Saju George, K.Vrinda Menon, C.Latha, B.Sunil, C.Sethulekshmi, Deepa Jolly

Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, India-680651
**Corresponding author*

A B S T R A C T

Present study was carried out in sewages of three different towns in Kerala, India to determine the occurrence of *Listeria* specific bacteriophages. Presence of the phages in an environment could be considered as an indication of the presence of its host. In this study, the phages were propagated using double agar layer method against the host organism *Listeria monocytogenes* (MTCC-1143). Out of 110 samples screened, 18 positive isolates were recovered from the sewages .The recovery status of the phages was found to be 16.36 %.

Keywords

Listeria monocytogenes, bacteriophage

Introduction

Bacteriophages are the natural enemies of bacteria. Recently, they have again moved into the focus of research interest, with respect to biocontrol of pathogenic bacteria as well as offerering tools for novel and effective separation technologies and diagnostics. In fact, bacteriophages present ideally suited means to control and detect *Listeria* cells in foods. *Listeria* phages can be isolated with relative ease from various environmental sources by the soft agar overlay method (Klumpp and Loessnor, 2013). Phages are most widely distributed and diverse entities in the biosphere and ubiquitously present which can be found in all reservoirs populated by bacterial hosts, such as soil, and sea water and the intestine of animals (Mc Grath and Van Sinderen, 2007). *Listeria monocytogenes* is a zoonotic food borne pathogen and is ubiquitous in nature. It has been recovered from dust, soil, water, sewage and decaying vegetation, including animal feed and silage from where the organisms enter the cycle of transmission. (Ikeh et al.,2010). The presence of this organism in environment such as sewages can become a source of infection to humans and animals. Therefore in this study bacteriophages were isolated for the early detection of pathogenic organism such as Listeria in the environment.

Materials and Methods

Sample collection: 110 samples (45ml

each) were collected using sterile plastic vials from sewages of corporation markets of three towns of Kerala. The details of samples collected from various place are shown in the table.

Host Bacteria: Standard cultures of *Listeria monocytogenes* (MTCC 1143), obtained from Microbial Type Culture collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The isolates were maintained in Brain Heart Infusion (BHI) broth with ten percent glycerol by subculturing at regular intervals and periodically tested for their purity, morphological and biochemical characteristics.

Isolation by double agar layer method:

The protocol for the isolation of sewage is based on double agar method as described by Adams (1959) with slight modifications and was carried out for a period of three days. On the first day, from the collected sewage sample, 10 millilitres was taken in sterile plastic centrifuge tube and was centrifuged at 5000rpm for 10 minutes. After centrifugation, the supernatant was taken and filtered through a syringe filter which is having a pore size of 0.45 µ. About nine millilitres of filtrate was taken and to this one millilitre of *Listeria* culture was added which was then incubated overnight at 37^0 C. To enhance the bacteriophage growth 10mM $CaCl_2$ and 0.5mM $MgSo_4$ were added to the above mixture and incubated at 37^0C for 24 hours. On the second day, the incubated sample was centrifuged at 5000 rpm for 10 minutes and the supernatant was filtered through 0.45µ syringe filter. From the filtrate one millilitre was taken in separate sterile test tube and to this two to three drops of three to six hours old *Listeria* culture was added along with three millilitres of one percent soft agar maintained at a temperature of 40- 45^0C. This mixture was then added to TSA plates and allowed to solidify. This plate was incubated at 37^0 C for 24 hours. On third day the plates were examined for plaque formation. The plaques were seen as a clear transparent zone which was circular in shape.

Results and Discussion

Out of the 110 samples, 18 samples were found to be positive for phages as they showed clean circular zones (complete inhibition) of the bacterial growth. Zones of 2mm diameter were taken as positive samples. It was then picked up using pasture pipette and stored in one millilitre phosphate buffer saline (PBS) containing one drop of chloroform (99 per cent). The recovery of phage from sewage samples was found to be 16.36 percent.

Bacteriophages are the natural enemies of bacteria. Recently, they have again moved into the focus of research interest, with respect to biocontrol of pathogenic bacteria as well as offerering tools for novel and effective separation technologies and diagnostics. In fact, bacteriophages present ideally suited means to control and detect Listeria cells in foods (Klumpp and Loessnor., 2013). The present study investigates the isolation of bacteriophages specific for *Listeria monocytogenes* from sewage. Out of the 110 sewage samples, 18 bacteriophages specific for *L.monocytogenes* were recovered, with a phage recovery of 16.36 per cent. Kim *et al*. (2008) could isolate a total of 14 *L.monocytogenes* specific bacteriophage from the sewages of a turkey processing plant, with a recovery status of 12.40 per cent, which was lower than the result of present study.

Table.1 Occurrence of bacteriophages in sewage samples

Source	No of Samples	Positive Samples	
		Number	Per cent
Thrissur	66	14	21.21
Kozhikode	22	-	-
Palakkad	22	4	18.18
Total	**110**	**18**	**16.36**

Loessner (1991) could isolate one *L*.monocytogenes specific bacteriophages (C722) from sewage samples which were in accordance with the present study as the phages were isolated from sewages. Jonczyk *et al.* (2011) suggested the influence of external factors such as presence of calcium and magnesium ions and also pore size of the filters used in isolation which might result in variation in the recovery status of the phages from the environment.

Phages are host specific and are seen in the environment where the hosts are abundant (Krone and Abedon., 2008). Similar results were obtained in the present study as 14 phage isolates were obtained from the same site (Thrissur and Palakkad) were *Listeria* spp. were isolated. According to Kim *et al.* (2008), the recovery status of *Listeria* spp. specific bacteriophage was 27 per cent as compared to 14 per cent for *L.monocytogenes* which is in accordance with the present investigation. More investigative studies are necessary for the species wise confirmation of the phages.

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