

Phage-Based Biocontrol of *Escherichia coli* Contamination in Non-Thermal Ready-to-Eat Shrimp Products at West Coast Frozen Foods

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DOI: [https://doi.org/10.63001/tbs.2026.v21.i04.S.I\(4\).pp92-97](https://doi.org/10.63001/tbs.2026.v21.i04.S.I(4).pp92-97)

KEYWORDS

Bacteriophage;
Escherichia coli; Ready-to-Eat Shrimp; Non-Thermal Treatment; Biocontrol

Article Info

Received : 14/08 /2026

Accepted :20/09/2026

Published: 07/10/2026

Abstract

Raw, ready-to-eat shrimps are susceptible to contamination by food-borne pathogens at various processing stages, from harvesting through packaging before export. As a result, these products are vulnerable to bacterial contamination. Among bacteria, *Escherichia coli* serves as an indicator of faecal contamination, and its presence in the products poses a public health risk. To reduce contamination, conventional methods such as thermal processing, freezing, or chemical antimicrobials are employed; however, these methods are often unsuitable, leaving chemical residues or affecting product quality. Thus, a safer, more efficient method that does not affect public health or the environment is needed. The bacteriophage plays a vital role in the fight against contamination due to its host specificity, self-replication and eco-friendliness. The applications of phage integrations cover many fields, especially in food processing due to their wide application. They have also been designed to fight food-borne pathogens, especially in fish products. This research aims to reduce *Escherichia coli* in the shrimp and water from the West Coast Frozen Foods Pvt Ltd treatment plant. The results show that using phages could significantly cut down bacterial contamination during production. As a result, this phage-based method could help produce safe seafood for consumers and support a sustainable global food system.

1.INTRODUCTION

Seafood is one of the major parts of the diet all around the world that provides a surplus amount of protein and minerals. Projections suggest that by 2030, the average per-person consumption of seafood will increase to 21.4 kg (Yan J, *et al.*,2024). Hence, the economic worth and variety of shrimp in food preparations make it more appealing than other

marine products (FAO, 2022). India has been the foremost exporter of shrimp, specifically black tiger shrimp (*Penaeus monodon*) and Vannamei shrimp (*Litopenaeus vannamei*), which contribute around 50-60% of the total export value. Most of the export materials are farm-raised and are often processed as block frozen/ Individually Quick Frozen (IQF) to preserve quality during international transport across

the United States, the European Union, and Japan. (Mohamed Hatha *et al.*, 2003).

The export of Shrimp products is administered by international food Safety and quality standards. The seafood exporters across India have to follow Codex Alimentarius guidelines, ISO certifications (ISO 9001, ISO 22000), HACCP protocols, and regulatory requirements set by the EU and FDA in order to process and export the product with the best quality for consumers to purchase. West Coast Frozen Foods exports its products to countries such as the United States, Europe, Japan, Russia, and the Middle East. So far, the company has a strong reputation for consistent product quality, timely deliveries, and adherence to global standards, earning trust in the seafood export industry. (Manual No. WCFPL/2024/HACCP-01 Revision Date 07th Dec 2025) (Pages 13 & 14).

Although they adhere to all regulations, there is a gap that allows bacteria to infiltrate the product through many phases, including harvesting, sorting, processing, freezing, storing and distribution (Heinitz *et al.*, 2000). *Acinetobacter*, *Serratia*, and *Pseudomonas* are common spoilage bacteria in seafood (Zhang J. *et al.*, 2024). Seafood is susceptible to contamination by hazardous bacteria such as *Salmonella typhimurium*, *Clostridium botulinum*, *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Vibrio vulnificus*, *Vibrio cholerae*, and *Vibrio parahaemolyticus*, which might pose a threat to human health (Littman *et al.*, 2020). Depending on the pathogen, this can result in a variety of health problems.

The presence of *Escherichia coli* is an indication that the surface is contaminated by faecal matter, which is likely to generate public health risk and Epidemics

(Cabral, J. P. S., 2010). The conventional method fails to control the *Escherichia coli* contamination in the Raw shrimp products, as the WHO (2020) states that the water can be a potential source of contamination for shrimp. Although the water used undergoes several treatments, shrimp still tend to exhibit the presence of undesired bacteria, which can lead to foodborne illnesses.

As a result, to mitigate the food-borne illnesses caused by the harmful contamination of food-borne pathogens, bacteriophages present multiple advantages compared to traditional microbial control methods. Phages are viruses that are engineered to attack certain hosts, infecting and killing only the bacteria they are meant to kill. They have attracted significant interest as a sustainable and environment friendly approach to control germs in several fields such as agriculture, healthcare and food processing (Garcia *et al.*, 2008). Phage-based treatments have proven to be beneficial in a variety of food systems; however, their use for *E. coli* control in raw, ready-to-eat shrimp is not widely explored (Hagens, S, & Loessner, M. J., 2010).

Thus, this study analyzes the efficacy of the bacteriophage on the *Escherichia coli* commonly found in the raw, ready-to-eat shrimp prepared at West Coast Frozen Foods. To this end, the isolation and characterization of the *E. coli* strains commonly present in shrimp and the assessment of the bacteriophage against the target

Hence, this study determines the effectiveness of the specific bacteriophage against *Escherichia coli* in the raw ready-to-eat shrimp prepared at the West Coast Frozen Foods. To this end, the research includes a number of key elements: isolation and characterisation of bacteriophages specific to *E. coli*

strains commonly found in shrimp, testing of their efficacy under conditions relevant to shrimp processing, and investigation of the practical feasibility for large-scale production.

2. MATERIALS AND METHODS

The *Escherichia coli* species prevalent within the water and frozen shrimp treated and untreated samples were enumerated using the plate count technique on Tryptone Bile X-glucuronide (TBX) agar according to ISO 16649-2 (ISO, 2001). As TBX is a selective agar, it enriches the *Escherichia coli* species more prominently, seen with β -glucuronidase activity producing blue-green colonies when incubated at $44 \pm 1^\circ\text{C}$ for 18–24 hours. The effectiveness of the phage in reducing *E. coli* populations was tested by monitoring and counting colonies isolated from the treated and untreated samples of frozen shrimp and water, respectively.

2.1 SAMPLE PLAN:

The water and frozen shrimp samples were obtained from two processing units (Unit-1 and Unit-2) and two types of samples were collected. The water samples were labelled as WS101 (Unit 1) and WS201 (Unit 2) while the shrimp samples were labelled as FS101 (Unit 1) and FS201 (Unit 2). Sampling was carried out over four consecutive days and four sets of samples were examined. Eight samples of each group, control and phage-treated groups were taken which makes a qualified evaluation of the microbiological parameters against the efficacy of the phage.

2.2 SAMPLE COLLECTION:

The samples were obtained using sterile sampling bottles under aseptic conditions to avoid any contamination. Water samples were obtained

directly from the processing plant, whereas frozen shrimp samples were obtained from the final processed packaged batches. Each sample was labelled with a code and transported to the laboratory in insulated ice boxes at a temperature of 4°C with care for ensuing microbiological and physicochemical evaluations.

2.3 HOMOGENIZATION

The frozen samples were defrosted at room temperature ($25^\circ\text{C} \pm 2^\circ\text{C}$) for one to two hours and then homogenized. The composite sample was accurately measured in a sterile manner, weighed 25 grams and placed in a stomacher bag. With this 225 mL sterile buffered peptone water. The sample was then mixed for 30 seconds in a stomacher to ensure that the sample was well mixed and homogenous. This method is required in order to get a consistent suspension of the material for analysis.

2.4 INOCULATION AND INCUBATION

The process of inoculation and incubation was carried out with careful aseptic techniques in order to prevent contamination. First, 1 ml of the homogenized sample was measured and added to two sterile petri dishes. Then 12 to 15 mL of sterile, cold Tryptone Bile X-glucuronide (TBX) agar was poured to each plate. The agar was chilled to $44\text{--}47^\circ\text{C}$ so that it was still fluid enough to mix with the sample without damaging the microorganisms. The plates were kept undisturbed until the agar solidified after the addition of the infected sample. It is vital to note that the pouring of agar should not be delayed longer than 15 minutes after inoculation, in order to prevent drying out or contamination of the sample. After the agar solidified, the plates were incubated at 44°C for 18 to 24 hours. This temperature was selected to favor the development of some specific

bacteria relevant to the analysis. After incubation, the dishes were observed for growth and colony development, which could subsequently be tested to determine the microbiological composition of the sample. Simultaneously, for the phage therapy process, 4 mL of concentrated phage was combined with 1000 mL of sterile water to prepare a diluted phage solution. This solution (10 mL) was carefully added to the sample of diluted *Escherichia coli* at room temperature. The sample was left at room temperature for 2 h, allowing time for phages to attach to and potentially infect any *Escherichia coli* present in the sample. After this incubation, samples were inoculated on TBX agar as described in the conventional procedure. This phase in this experiment is to determine the efficacy of phage therapy in reducing *Escherichia coli* contamination in the samples by inoculating the samples under controlled conditions to promote further interaction between phages and *Escherichia coli*, to create a therapeutic effect.

3. RESULT AND DISCUSSION

The untreated water samples tend to show the presence of *Escherichia coli* with the help of TBX agar used. According to the ISO method, typical blue/green colonies were noted and enumerated. All the samples collected from the selected area of both unit 1 and unit 2 within four consecutive days indicated the presence of *Escherichia coli*, but within the accessible limit as given by Export Inspection Council (EIC/F & FP, 2012). Whereas results obtained from the water samples treated with phage show a significant reduction in the number of *Escherichia coli* species. Figure 1 clearly shows the difference between untreated and treated water samples. Before phage treatment, the bacterial count

ranges from 50 to 90 CFU/ml. After the phage treatment, there is a rapid reduction in the number of *Escherichia coli*, which then ranges from 5 to 15 CFU/ml.

In the case of the frozen shrimp sample, it indicated the presence of *Escherichia coli*, as the water used for cleansing the shrimp was untreated, which was directly taken from the water treatment plant. Whereas the frozen sample processed beforehand with the treated water has a limited number of *Escherichia coli* species (Table 2). Hence, this significantly implies that the usage of bacteriophage in the water treatment plant can reduce the bacterial count, which, on further usage before the shrimp is being processed, can further reduce the limited bacterial count.

Figure 3 significantly depicts the difference between the untreated and the treated shrimp samples. Before the phage treatment the bacterial count ranges between 300 to 500 CFU/gm. But once the treatment of phage in the water sample there has been a rapid reduction in the number of *Escherichia coli* species that in turn ranges between 30 to 70 CFU/mg.

From table 3 the impact of bacteriophage treatment on bacterial colony-forming units (CFU) in water and shrimp samples can be speculated. The control CFU levels for water samples range from 54 to 84, while for shrimp samples, they range from 335 to 475. Post-treatment with bacteriophages, substantial reductions in bacterial counts are observed in both water and shrimp samples. In water samples, the percentage reduction varies between 73.87% and 93.84%, demonstrating the effectiveness of phage treatment, with the highest reduction (93.84%) occurring at a control CFU of 73 and the lowest (73.87%) at a control CFU of 56. Similarly, shrimp

samples exhibit a percentage reduction ranging from 82.09% to 95.00%. The highest reduction (95.00%) is recorded at a control CFU of 420, while the lowest reduction (82.09%) is observed at a control CFU of 335 (Table 3).

Generally, the data suggest that the bacteriophages are effective in reducing the bacterial counts in both environments, with slightly higher percentage reduction in the shrimp samples than in water samples. The variation in % decrease may be attributed to the variances in the baseline bacterial numbers, phage concentration, or environmental circumstances.

Both samples exhibit good reductions in the range of 80% for eight measurements as shown in Figure 3. In the water sample we can observe a clear drop in the 5th measurement, where the reduction percentage goes below 80% and then recovers in the next measures. The shrimp sample shows a more steady pattern with decreases around 85-90% with a little drop around measurement 8. The phage treatment seems to be very efficient in reducing the targeted *Escherichia coli* species in general in the water and shrimp samples.

4. CONCLUSION

This study shows the pertinency of bacteriophage therapy as a novel approach to improve food safety in raw ready-to-eat food products. The use of Phages significantly reduces bacterial contamination and is a safe alternative to the conventional process. The successful elimination of *Escherichia coli* contamination in shrimp points to the potential for phage application at the industrial level, especially in non-thermal items such as sushi and sashimi. In addition, data on microbiological load and sources of contamination are the basis for the establishment of

standards and effective control methods in the seafood industry. Phage therapy could revolutionise the way the seafood industry controls germs in the long term, making food safer for consumers and greener for the global food chain. Bacteriophage therapy has the potential to totally change food safety if the public continues to accept it and regulators approve it. Ongoing research and development will certainly allow for its wider use, which will improve public health and safer food systems.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this manuscript, the authors used QuillBot to assist with grammar and language improvement and Google Gemini to assist in preparing the graphical abstract. After using these tools, the authors reviewed and edited the outputs as necessary and take full responsibility for the accuracy, originality, and content of the publication.

Declaration of competing interests

The authors have no conflicts of interest to declare.

Data availability

Data will be made available on request.

Acknowledgements

The authors sincerely thank West Coast Frozen Foods Pvt. Ltd., Surat, Gujarat, for granting permission and providing the necessary support to carry out this research work. The authors also express their sincere gratitude to St. Xavier's College, Palayamkottai, for granting permission and providing institutional support to conduct this research.

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