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# Establishment of PCR System for Rapid Detection of Four Kinds of Common Aquatic-Food Pathogens

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***Abstract:***

In this paper, using polymerase chain reaction (PCR) detection method, with sets of primers from the *tlh*, *tdh*, *trh*, *hly*, *inv* A, *rfbe*, *stx* 1, *stx* 2 *of Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Salmonella* and *Escherichia Coli* were designed to establish a novel method for rapid detection of common aquatic-food pathogens. The results showed that the new established PCR system was 100% specificity and using this method was capable of detecting a minimum of $8.28\times10^{1}$ CFU/mL for genomic DNA. This method was directly applied in the aquatic samples and the detection limit was be $4\times10^{3}$ CFU/mL, $2.14\times10^{3}$ CFU/mL, $8\times10^{4}$ CFU/mL and $1.07\times10^{3}$ CFU/mL respectively. It could be detected in 3hours. It reveals that a rapid PCR technique for the detection of common aquatic-food pathogens was established successfully.

***Keywords:*** *PCR, Pathogens, Rapid detection*

## I. INTRODUCTION

Global food safety incidents have occurred frequently and have become an important factor affecting public health in recent years. Food safety issues have increasingly become a hot spot of social concern. Food-borne pathogens are the main factor that endangers food safety and human health [1-3]. Explosive food poisoning caused by edible aquatic products also frequently occurs. Common pathogens detected in aquatic products include: *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Vibrio cholerae*, *Vibrio vulnificus*, *Vibrio alginolyticus*, *Salmonella*, and *large intestine Bacillus*, etc. These pathogenic bacteria can cause severe vomiting, diarrhea, headache, and fever. In severe cases, dehydration, body cramps, confusion, blood pressure and other shock symptoms are often life-threatening. Therefore, establishing an effective detection system for pathogenic microorganisms in aquatic products, and quickly and accurately completing the detection of pathogenic bacteria in food has become the key to ensuring food safety and preventing disease transmission.

These routine detection methods for pathogenic microorganisms require multiple steps such as separation and culture, biochemical tests, and serological tests. The detection cycle is long, the operation is cumbersome, and the sensitivity is low. Moreover, only one pathogen can be detected at a time. Its sensitivity is also affected to a certain extent by the interference of miscellaneous bacteria, there are certain

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false negatives, and the detection of pathogenic microorganisms that are difficult to be artificially cultivated cannot be detected, which is far from meeting the requirements of modern detection.

With the development of molecular biology, detection technology established on the basis of molecular biology has gradually become a detection method for pathogenic bacteria in food. Such as DNA fingerprinting, immune capture, typing technology, colony hybridization, PCR, real-time quantitative PCR, gene chip, etc., have also shown its unique advantages in the detection of food-borne pathogens. Among them, the PCR method has the advantages of high efficiency, high yield, low cost, and fast speed, and has good operability. It has been applied in the detection of a variety of pathogenic microorganisms [4-8].

This article focuses on the common pathogenic microorganism *Vibrio parahaemolyticus* thermotoxin *tlh* gene and its virulence genes *trh* and *tdh* genes, *Listeria monocytogenes* hemolysin *hly* gene, *Salmonella invA* gene and *Escherichia coli* O157 *rfbe*, *stx 1* and *stx 2* genes are used as target genes to design primers to establish a PCR reaction system that can quickly and integratedly detect the four common pathogenic microorganisms at the same time. The method is evaluated in terms of specificity and sensitivity, and it is directly applied to the actual detection of aquatic product samples. At the same time, this study also laid a certain foundation for the application of PCR technology to the efficient detection of other pathogens, and played an active role in the early warning, monitoring, analysis and investigation of pathogens.

## II. MATERIALS AND METHODS

### 2.1 Material

#### 2.1.1 Strains

All 58 standard strains used in this experiment were provided by Wuhan University of Technology. Including *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella*, *Escherichia coli*, *Yersinia enteroconjunctivitis subsp.* and *Pseudomonas aeruginosa*.

#### 2.1.2 Main instruments and equipment

YXQ-LS-75S II vertical pressure steam sterilizer (Shanghai Boxun Industrial *Co., Ltd.* Medical Equipment Factory); ZHWY-2102 double-layer large-capacity full temperature constant temperature shaker (Shanghai Zhicheng Analytical Instrument Manufacturing *Co., Ltd.*); HC-2518R High-speed refrigerated centrifuge (Anhui Zhongke Zhongjia Scientific Instrument *Co., Ltd.*); DYY-6C electrophoresis instrument (Beijing Liuyi Instrument Factory); Tocan240 gel imaging system (Tocan); TC-96HBPCR amplification instrument (Hangzhou Bori Technology) *Co., Ltd.*); UV-7504 single beam ultraviolet-visible spectrophotometer (Shanghai Xinmao Instrument *Co., Ltd.*); DS-1 high-speed tissue masher (Shanghai Jingke Industrial *Co., Ltd.*).

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2.1.3 Reagents and media

Bacterial genomic DNA rapid extraction kit (silica gel membrane spin column method), Bst DNA polymerase, 10×ThermoPol Reaction buffer, and 10mM *d*NTPs were purchased from Guangzhou Dongsheng Biotechnology *Co., Ltd.*; fresh kelp was purchased from a local supermarket.

Selective medium for *Vibrio parahaemolyticus*: provided by Qingdao Haibo Biological Company.

*E. coli* O157 selective medium: provided by CHROmagar.

*Salmonella* selective medium (SS): Provided by Shanghai Kexing Trading *Co., Ltd*.

*Listeria monocytogenes* selective medium: peptone 15.0 g, yeast extract powder 5.0 g, agar 15.0 g, sodium chloride 5.0 g, pigment 2.3 g, inhibitor 5.0 g, deionized water (plate) 1 L.

3% sodium chloride alkaline peptone water: sodium chloride 5 g, potassium nitrate 0.1 g, peptone 20 g, 1 L distilled water, heated to dissolve.

LB broth liquid medium: 21 g of LB broth, 1 L of distilled water, heated to dissolve.

LB broth solid medium: 1 L LB broth liquid medium plus 15 g technical agar powder.

*Listeria monocytogenes* selective medium, LB broth, and technical agar powder were all purchased from Guangdong Huankai Microbial Technology *Co., Ltd*.

2.2 Method

2.2.1 Cultivation of strains

21 g LB broth was dissolved in 1 L of distilled water, heated to dissolve, and then aliquoted. Sterilize at 121°C to obtain a basal medium. In the ultra-clean workbench, add 5% of the inoculum amount to the sterilized culture medium with the mother liquor of the bacterial species, and cultivate for 12 h at 37°C on a shaker at 150 rpm.

2.2.2 Screening of test strains

58 standard strains were inoculated into *Vibrio parahaemolyticus* selective medium, *Salmonella* selective medium, *Listeria monocytogenes* selective medium and *Escherichia coli* O157 selective medium, 37°C, static culture 48 h. Screen out the four strains required for the test.

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2.2.3 Extraction of DNA template

Take 1 mL of the bacterial suspension cultured overnight and add it to a 1.5 mL sterile centrifuge tube, centrifuge at 12000 rpm for 2 min, aspirate the supernatant as much as possible, add 50 μL of eluent TE, mix well, and then boil in a boiling water bath for 5 min, centrifuge at 12000 rpm 5 min, take the supernatant and store at -20°C. 1.2 Method

2.2.4 Primer design and synthesis

*Vibrio parahaemolyticus tlh* gene, *Listeria monocytogenes* hemolysin *hly* gene, *Salmonella InvA* gene and *E. coli* O157 *rfbe*, *stx 1* and *stx 2* gene sequences were searched through the GenBank database (http://www.ncbi.nlm.nih.gov/nuccore /472320310). LAMP primers were designed using PrimerExplorer V4 primer design software. Two outer primers F3 and B3 were used to test the PCR system. The designed primers were compared with the nucleic acid sequences in the Genbank database to ensure the high specificity of the primers for the four food-borne pathogenic microorganisms. The primer sequence is shown in Table I.

**Table I. The sequence of PCR primers for three kinds of common foodborne pathogens**

| **Strains** | **Target gene** | **Primer (5'—3')** | **Fragment length** |
|---|---|---|---|
| *Vibrio parahaemolyticus* | *tlh* | TLF: CGCTGACAATCGCTTCTCAT<br>TLB: GTTCTTCGCTTTGGCAATGT | 214 bp |
| | *tdh* | TDF: GTTCGAGATACAACTTTTAATACCA<br>TDB: CGTGCTTATAGCCAGACAC | 211 bp |
| | *trh* | TRF: ACAACAATAAAAACTGAATCACC<br>TRB: GACCGTTGAAAGGCCATC | 216 bp |
| *Listeria-monocytogenes* | *hly* | HLF: GGAGGMTACGTTGCTCAA<br>HLB: AAGCTAAACCAGTGCATTC | 211 bp |
| *salmonella* | *inv*A | INF: GAACGTGTCGCGGAAGTC<br>INB: CGGCAATAGCGTCACCTT | 284 bp |
| *Escherichia coli O157* | *rfbe* | RFF: AACAGTCTTGTACAAGTCCA<br>RFB: GGTGCTTTTGATATTTTTCCG | 159 bp |

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| | | |
|---|---|---|
| *stx* 1 | S1F: TGTTGGAAGAATTTCTTTTGGA<br>S1B: GCTAATAGCCCTGCGTATC | 232bp |
| *stx* 2 | S2F: TCGGTGTCTGTTATTAACCA<br>S2B: TGGAAACCGTTGTCACAC | 320bp |

2.2.5 Establishment of PCR reaction system

The PCR amplification system in this experiment includes 50 μL, 25 μL 2×TaqPCRMasterMix (with dye), 2 μL primer F, 2 μL primer B, 2 μL template DNA, 19 μL dd$H_2O$. The reaction program was set as 94 C-5 min, 94 C-30 s, 55°C-30 s, 72°C-30 s, 30 cycles; 72°C-10 min. After the reaction, the results were analyzed by 2% agarose gel electrophoresis.

2.2.6 PCR reaction specificity and sensitivity test

All test strains selected in the 1.2.2 experiment were used in the amplification reaction, and the corresponding primers were added. The reaction products were analyzed by 2% agarose gel electrophoresis to evaluate the specificity of the method. Water was used as a template as a negative control. Each primer was tested with 20 different strains. *Vibrio parahaemolyticus* was used as the test strain to determine the sensitivity of the reaction system, and the concentration was measured with a single-beam UV-Vis spectrophotometer. The DNA stock solution was diluted by a factor of 10 to $10^{-1}$~$10^{-9}$, and each Dilute 2μL of the gradient DNA solution as an amplification template for LAMP detection. Water was used as a template as a negative control.

2.2.7 Simultaneous PCR test with multiple detection targets

An integrated system for simultaneous detection of 8 targets was developed. By amplifying 58 standard strains in the same PCR program against their 8 targets, the PCR machine is 96-well, and 12 strains can be detected at one time to investigate the specificity of integrated detection of strains.

2.2.8 Sensitivity test of artificially contaminated seafood samples

Four strains were used as test strains to determine the sensitivity of simulated samples. 25 g of kelp was added to 225 mL of 3% sodium chloride alkaline peptone water and homogenized with a DS-1 high-speed tissue masher for about 60 s to prepare kelp homogenate. The bacterial solution to be inoculated was diluted isocratically. Aseptically, 1 mL of each gradient-diluted pure bacterial culture solution of $10^{-1}$~$10^{-9}$ were added to 9 mL of kelp homogenate and mixed. 1 mL of artificially contaminated dilution gradient homogenates were put into 1.5 mL sterile centrifuge tubes, centrifuged at 3000 rpm for 5 minutes. The supernatant was transferred to another 1.5 mL sterile centrifuge tube, and centrifuged at 12000 rpm for 5 minutes, Aspirate the supernatant as much as possible, resuspend the pellet with 1 mL of

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0.9% sterile saline, and extract DNA for LAMP amplification. The uncontaminated kelp homogenate was used as a template for amplification as a negative control.

## III. DISCUSSION

### 3.1 Detection of Specificity and Sensitivity

The results showed that the *Vibrio parahaemolyticus* primers designed with *tlh*, *tdh*, and *trh* genes were positive for 8 strains of *Vibrio parahaemolyticus*, while the amplification results of the other 12 contrast strains were negative (as shown in Figure 1). *Listeria monocytogenes* primers designed with *hly* gene were positive for 10 strains of *Listeria monocytogenes* and negative for other control strains such as *E. coli* and *Salmonella* (as shown in Figure 2). *Salmonella* primers designed with *invA* gene were positive for 9 strains of *Salmonella* and 6 strains of *Escherichia coli* O157, negative for the other 11 comparative strains (as shown in Figure 3 and Figure 4). The six pairs of primers for the four strains have a detection rate of 100% for the target strain, and the amplification results for non-target strains are all negative. The research results show that the specificity of the detection method is 100%.

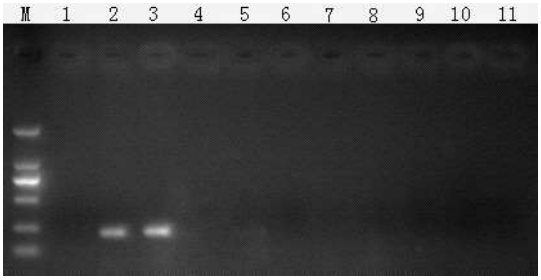


Fig.1 Agarose gel electrophoresis for specificity test of *Vibrio parahemolyticus*. M- DS2000 mark; 1-Negative control; 2, 3-*Vibrio parahemolyticus*; 4-*Salmonella subsp*.; 5, 6-*Staphylococcus aureus*; 7, 8-*Escherichia coli*; 9~11-*Listeria monocytogenes*

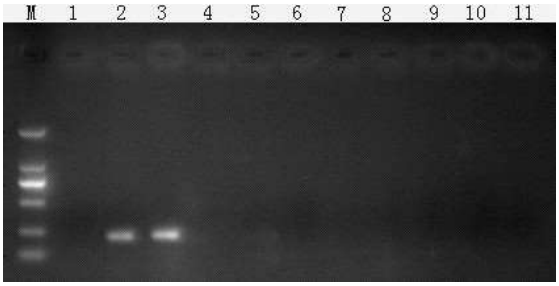


Fig.2 Agarose gel electrophoresis for specificity test of *Listeria monocytogenes*. M-DS2000 mark; 1-Negative control; 2, 3-*Listeria monocytogenes*; 4-*Vibrio parahemolyticus*; 5-*Escherichia coli*; 6-*Staphylococcus aureus*; 7-*Salmonella subsp*.; 8-*Pseudomonas aeruginosa*; 9-Y*ersinia enterocolitica subsp*; 10-*Bacillus cereus*

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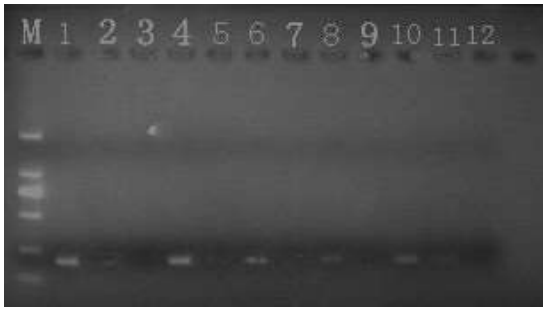


Fig.3 Agarose gel electrophoresis for specificity test of *Salmonella*. M-DS2000 mark; 1, 4, 6, 8, 10-*Salmonella*; 2-*Bacillus cereus*; 3-*Listeria monocytogenes*; 5-*Vibrio parahemolyticus*; 7, 9-*Escherichia coli*; 10-*Yersinia enterocolitica subsp*; 12-*Pseudomonas aeruginosa*

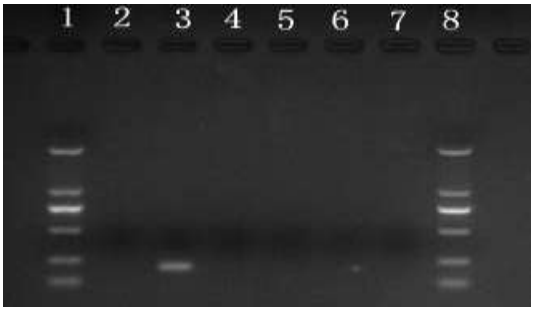


Fig.4 Agarose gel electrophoresis for specificity test of *Escherichia Coli* O157. 1, 8-DS2000 mark; 2-Negative control; 3-*Escherichia Coli*O157; 4-*Vibrio parahemolyticus*; 5-*Salmonella*; 6-*Staphylococcus aureus*; 7-*Bacillus cereus*

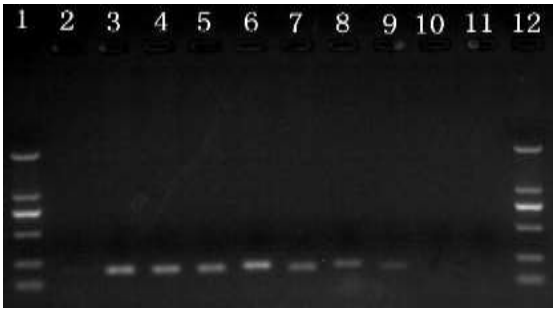


Fig.5 Agarose gel electrophoresis for sensitivity test by PCR. 1, 12- DS2000 mark; 2-Negative control; 3-$2.07\times10^{8}$ CFU /mL; 4-$2.07\times10^{7}$ CFU /mL; 5-$2.07\times10^{6}$ CFU /mL; 6-$2.07\times10^{5}$CFU/mL; 7-$2.07\times10^{4}$ CFU /mL; 8-$2.07\times10^{3}$ CFU /mL; 9-$2.07\times10^{2}$ CFU /mL; 10-$2.07\times10^{1}$ CFU /mL; 11-$2.07\times10^{0}$ CFU /mL

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3.2 Multi-Target Simultaneous PCR Detection

12 strains of bacteria can be detected at the same time for eight targets through a PCR machine. The results showed that the 58 standard strains selected in this experiment were detected at a rate of 100% by using a PCR program established in this experiment, indicating that the established simple PCR system can be used for simultaneous detection of multiple strains at multiple sites. In the future new generation of 324-hole PCR instrument, it will be possible to simultaneously detect 8 targets of 40 aquatic products, greatly improving the detection efficiency.

3.3 Sensitivity Detection of Artificially Contaminated Seafood Samples

The standard strain of *Vibrio parahaemolyticus* ATCC 17802 to be inoculated was counted on the plate and its concentration was $4\times10^7$ CFU/mL. Take a 10-fold dilution of $10^{-1}\sim10^{-9}$ 1 mL of bacterial solution into 9 mL of kelp homogenate, and mix it as the original sample of artificially contaminated seafood, so that the concentration of *Vibrio parahaemolyticus* in the original sample of artificially contaminated seafood is $4\times10^7$ CFU/mL (9 mL kelp homogenate is equivalent to 1 g kelp). After artificial contamination, 1 mL of kelp homogenate was taken to extract DNA, and PCR amplification was performed. The PCR detection results of artificially contaminated seafood samples are shown in Figure 6. When the bacterial content in artificially contaminated seafood samples is $4\times10^6\sim4\times10^3$ CFU/mL, amplification reaction occurs and amplified fragments are generated. When the concentration is $4\times10^2$ CFU/mL, no fragments are produced and amplification reaction does not occur. Therefore, it is determined that the detection limit of this method for direct detection of *Vibrio parahaemolyticus* in food samples is $4\times10^3$ CFU/mL.

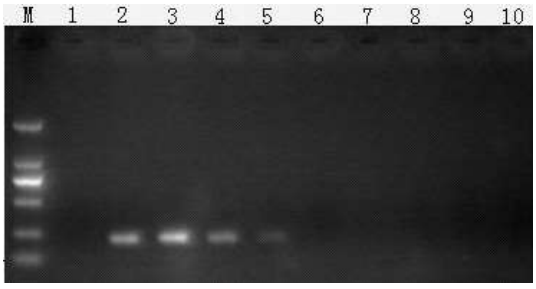


Fig.6 Limit test of artificially *Vibrio parahaemolyticus* polluted aquatic-food samples by PCR. M- DS2000 mark; 1-Negative control; 2-$4\times10^6$ CFU/mL; 3-$4\times10^5$ CFU/mL; 4-$4\times10^4$ CFU/mL; 5-$4\times10^3$ CFU/mL; 6-$4\times10^2$ CFU/mL; 7-$4\times10^1$ CFU/mL; 8-$4\times10^0$ CFU/mL; 9-$4\times10^{-1}$ CFU/mL; 10-$4\times10^{-2}$ CFU/mL.

The standard strain of *Listeria monocytogenes* ATCC 19118 to be inoculated was counted on the plate and its concentration was $2.14\times10^9$ CFU/mL. Take a 10-fold dilution of $10^{-1}\sim10^{-9}$ 1 mL of bacterial solution into 9 mL of kelp homogenate, and mix it as the original sample of artificially contaminated seafood, so that the *listeria monocytogenes* in the original sample of artificially contaminated seafood is $2.14\times10^9$ CFU/mL (9 mL kelp homogenate is equivalent to 1 g kelp). After artificial contamination, 1 mL

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of kelp homogenate was taken to extract DNA, and PCR amplification was performed. The PCR detection results of artificially contaminated seafood samples are shown in Figure 7. When the bacterial content in artificially contaminated seafood samples is $2.14\times10^{9}$～$2.14\times10^{3}$ CFU/mL, amplification reaction occurs and amplified fragments are generated. When the concentration is 2.14 when $\times10^{2}$ CFU/mL, no fragments are produced, and the amplification reaction does not occur. Therefore, it is determined that the detection limit of this method for direct detection of *Vibrio parahaemolyticus* in food samples is $2.14\times10^{3}$ CFU/mL.

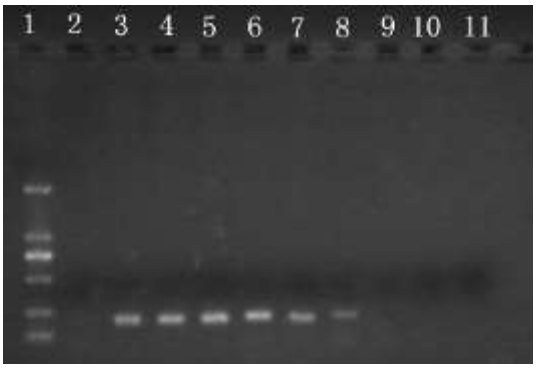


Fig.7 Limit test of artificially *Listeria monocytogenes* polluted aquatic-food samples by PCR. 1- DS2000 mark; 2-Negative control; 3-$2.14\times10^{9}$ CFU/mL; 4-$2.14\times10^{8}$ CFU/mL; 5-$2.14\times10^{7}$ CFU/mL; 6-$2.14\times10^{6}$ CFU/mL; 7-$2.14\times10^{5}$ CFU/mL; 8-$2.14\times10^{4}$ CFU/mL; 9-$2.14\times10^{3}$ CFU/mL; 10-$2.14\times10^{2}$ CFU/mL; 11-$2.14\times10^{1}$ CFU/mL

The standard Salmonella strain ATCC 29269 to be inoculated has a concentration of $8\times10^{7}$ CFU/mL after plate counting. Take a 10-fold dilution of $10^{-1}$～$10^{-9}$ 1 mL of bacterial solution into 9 mL of kelp homogenate, and mix it as the original sample of artificially contaminated seafood, so that the listeria monocytogenes in the original sample of artificially contaminated seafood is $8\times10^{7}$ CFU/mL (9 mL kelp homogenate is equivalent to 1 g kelp). After artificial contamination, 1 mL of kelp homogenate was taken to extract DNA, and PCR amplification was performed. The PCR detection results of artificially contaminated seafood samples are shown in Figure 8. When the bacterial content in artificially contaminated seafood samples is $8\times10^{7}$～$8\times10^{4}$ CFU/mL, amplification reaction occurs and amplified fragments are generated. When the concentration is 8 when $\times10^{3}$ CFU/mL, no fragments are produced, and the amplification reaction does not occur. Therefore, it is determined that the detection limit of this method for direct detection of *Vibrio parahaemolyticus* in food samples is $8\times10^{4}$ CFU/mL.

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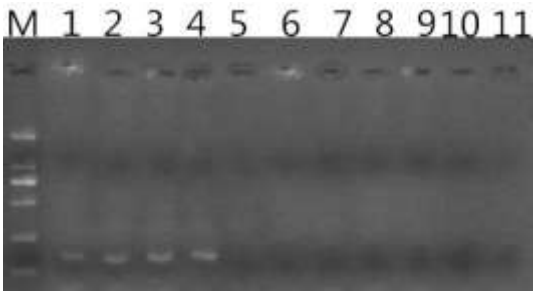


Fig.8 Limit test of artificially *Salmonella* polluted aquatic-food samples by PCR. M- DS2000 mark; 1-$8\times10^{7}$ CFU/mL; 2-$8\times10^{6}$ CFU/mL; 3-$8\times10^{5}$ CFU/mL; 4-$8\times10^{4}$ CFU/mL; 6-$8\times10^{3}$ CFU/mL; 7-$8\times10^{2}$ CFU/mL; 8-$8\times10^{1}$ CFU/mL; 9-$8\times10^{0}$ CFU/mL; 10-$8\times10^{-1}$ CFU/mL; 11-Negative control

The standard strain of *Escherichia coli* ATCC 43895 to be inoculated was counted on the plate, and its concentration was $1.07\times10^{9}$ CFU/mL. Take a 10-fold dilution of $10^{-1}\sim10^{-9}$ 1 mL of bacterial solution into 9 mL of kelp homogenate, and mix it as the original sample of artificially contaminated seafood, so that the *listeria monocytogenes* in the original sample of artificially contaminated seafood is $1.07\times10^{9}$ CFU/mL (9 mL kelp homogenate is equivalent to 1 g kelp). After artificial contamination, 1 mL of kelp homogenate was taken to extract DNA, and PCR amplification was performed. The PCR detection results of artificially contaminated seafood samples are shown in Figure 9. When the bacterial content in artificially contaminated seafood samples is $1.07\times10^{9}\sim1.07\times10^{3}$ CFU/mL, amplification reaction occurs and amplified fragments are generated. When the concentration is $1.07\times10^{2}$ CFU/mL, no fragments are produced, and the amplification reaction does not occur. Therefore, it is determined that the detection limit of this method for direct detection of *Vibrio parahaemolyticus* in food samples is $1.07\times10^{3}$ CFU/mL.


Fig.9 Limit test of artificially *Escherichia Coli* polluted aquatic-food samples by PCR. 1, 12- DS2000 mark; 2-Negative control; 3-$1.07\times10^{9}$ CFU/mL; 4-$1.07\times10^{8}$ CFU/mL; 5-$1.07\times10^{7}$ CFU/mL; 6-$1.07\times10^{6}$ CFU/mL; 7-$1.07\times10^{5}$ CFU/mL; 8-$1.07\times10^{4}$ CFU/mL; 9-$1.07\times10^{3}$ CFU/mL; 10-$1.07\times10^{2}$ CFU/mL; 11-$1.07\times10^{1}$ CFU/mL

Conventional isolation culture and biochemical identification are time-consuming and have low sensitivity, prone to false detections and missed detections, which are not conducive to the rapid diagnosis

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and treatment of food-borne emergencies. Compared with traditional culture methods and immunological methods, such as enzyme-linked immunosorbent assay and immunofluorescence, PCR technology has stronger specificity, higher sensitivity, and easier and faster operation. It has been used in the detection of foodborne disease pathogens. It is widely used [9-10].

This article focuses on the four common pathogenic microorganisms in aquatic products: *Vibrio parahaemolyticus, Listeria monocytogenes* and *Salmonella*, and *Escherichia coli* O157. Primers are designed for their specific and highly conserved genes, and multi-target simultaneous PCR is performed. Detection and research results show that the amplification results of the target strains are all positive, the specificity is 100%, and the sensitivity is high. The detection limit of genomic DNA is about $8.28\times10^1$ CFU/mL, which is directly sensitive to aquatic product samples. They are $4\times10^3$ CFU/mL, $2.14\times10^3$ CFU/mL, $8\times10^4$ CFU/mL and $1.07\times10^3$ CFU/mL, which are similar to the PCR method used by the predecessors for detecting only one strain [11-12], indicating that the method has strong practical value.

This research systematically analyzes the PCR detection and analysis methods of four common pathogenic microorganisms in aquatic products, evaluates their specificity and sensitivity, and simulates the real food system for each bacteria, and the practicality of the method Perform detection; the amplified fragments selected in this study are small, and each cycle requires a short time. Each PCR amplification program is only 30 s, and the designed eight pairs of primers are similar in length, so the same PCR annealing temperature can be set, All target strains are amplified under the same PCR program at the same time to realize the integrated simultaneous detection of multiple strains; the results of this test show that the established detection method has strong specificity and high sensitivity, and it can be detected in food systems. The high sensitivity of this method indicates that this method has a certain practical value; the eight pairs of primers designed in this experiment are highly specific and targeted, and can be used as reference primers for the detection of several bacteria by other detection methods; The target is highly specific, and the target can be extended to other detection methods, such as real-time fluorescent quantitative PCR detection method, multiplex PCR and loop-mediated isothermal amplification (LAMP) methods [13-15].

## IV. CONCLUSION

The rapid detection method established in this article is more conducive to the realization of integrated synchronous detection, and has strong practical significance and promotion value. It is of great significance for improving the level of food hygiene, ensuring food safety and promoting the development of food international trade.

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