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Selective endpoint visualized detection of *Vibrio parahaemolyticus* with CRISPR/Cas12a assisted PCR using thermal cycler for on-site application

Mengyao Zhang[a], Chengzhi Liu[b,c], Ya Shi[b,c], Jun Wu[d], Jian Wu[a,*], Huan Chen[b,c,**]

[a] College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, 310058, China
[b] Key Laboratory of Microbiol Technology and Bioinformatics of Zhejiang Province, Zhejiang Institute of Microbiology, Hangzhou, 310012, China
[c] NMPA Key Laboratory for Testing and Risk Warning of Pharmaceutical Microbiology, Zhejiang Institute of Microbiology, Hangzhou, 310012, China
[d] Lin'an Center for Disease Control and Prevention, Lin'an, 311300, China

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ABSTRACT

Vibrio parahaemolyticus is a major cause of seafood-associated food poisoning. It is of great significance to develop an accurate, simple and cost-effective method to identify infected seafood, especially for on-site application. Polymerase chain reaction (PCR) remains the golden standard for nucleic acid detection. But traditional methods heavily reply on sophisticated instrument and specialized operators, which limits the application for on-site detections. Here we developed a novel, specific and visualized detection method for PCR based on CRISPR/Cas12a system. On a low-cost thermal cycler, amplification reaction can be conducted easily. The CRISPR/Cas12a system was specifically designed to evaluate amplicons, eliminating false positive results. Besides the negative samples remained colorless, the positive samples generated obvious green fluorescence, which could be easily distinguished by the naked eye using a homemade UV device. The presented detection method was verified by detecting shrimp samples. The limit of detection is 1.02×10^2 copies/μL. This presented method provided a new strategy for specific endpoint detection of PCR and advanced its application in field for food safety assurance.

1. Introduction

Vibrio parahaemolyticus, a halophilic estuarine bacterium, is a major cause of seafood-associated food poisoning [1]. Eating *V. parahaemolyticus* infected seafood may lead to gastroenteritis, wound infection, and septicemia, which is harmful to health [2]. Consequently, it is vital to identify *V. parahaemolyticus* infected seafood to guarantee food safety for both food industry and the individual. Generally, diagnostics is the first step to determine the cause of symptoms for either pathology or food safety assurance. To effectively propose solving strategies for food safety monitoring, early detection is indispensable and should be rapid, specific and sensitive. With the development of modern society and technology, there is a growing demand for on-site detection because it provides portable, rapid, sensitive and user-friendly detection in non-laboratory and low-resource areas [3].

Nucleic acids are essential for any form of life because they all carry specific genes which could provide accurate identification to distinguish from others [4]. Consequently, the molecular analysis, aiming to detect specific nucleic acid sequences, has been the most common method in detection [5,6]. The polymerase chain reaction (PCR) is the earliest technology in molecular analysis. Although isothermal amplification has emerged as a promising alternative technique, PCR is still a powerful tool and remains the most popular amplification technology that is applied in various fields [7–9]. However, PCR usually requires bulky, expensive instrument and skilled operators, which might be unaffordable in low-resource areas. Currently, there are several portable and relative cheaper PCR thermal cyclers commercialized on the market (e.g., Longgene, Thermo Fisher Scientific, Bio-Rad). They claim to have a well-performance uniformity of temperature, guaranteeing the same PCR results with those of Real-time PCR Systems. This helps advance the potential on-site application for PCR.

In on-site detection, visualization of results is beneficial and friendly for users. In the presence of DNA intercalating dyes (e.g., SYBR Green I, SYTO 9) or colorimetric indicators, there tends to be an apparent distinction in color between positive and negative samples [10–14]. But these detection methods usually do not relate to the sequence of the

* Corresponding author.
** Corresponding author. Key Laboratory of Microbiol Technology and Bioinformatics of Zhejiang Province, Zhejiang Institute of Microbiology, Hangzhou, 310012, China.
E-mail addresses: wujian69@zju.edu.cn (J. Wu), chenhuan7809@gmail.com (H. Chen).

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target DNA, which means the specificity mainly depends on the inherent selectivity of amplification process. False positive results might occur due to non-specific amplification or primer dimers. This increases the requirement and difficulty in the optimization of PCR system. Thus, specific endpoint detection is supposed to carry out, ensuring the accuracy of PCR in simple sample treatment and amplification.

The clustered regularly interspaced short palindromic repeats (CRISPR) system, a component of bacterial adaptive immune systems, has been an emerging powerful genome editing tool in the past few years [15–18]. The CRISPR-associated proteins (CRISPR-Cas) showed precise double-stranded DNA (dsDNA) cleavage activity due to the two catalytic domains, RuvC and HNH [19,20]. Among these proteins, CRISPR-Cas12a (Cpf1) is one kind of nucleases that was reported to perform dsDNA cleavage under the guidance of the CRISPR RNA (crRNA) by utilizing a single RuvC catalytic domain[21]. The Cas12a enzyme recognizes a short T-rich protospacer-adjacent motif (PAM) and then catalyzes crRNA maturation to form an enzyme-RNA complex, generating a PAM-distal dsDNA break [22]. After the crRNA binds with a complementary DNA strand, the cleavage activity of this RNA-guided enzyme could be triggered, cleaving indiscriminate single-stranded DNA (ssDNA) [23]. Chen et al. created a DNA endonuclease-targeted CRISPR trans reporter (DETECTR) for specific and visualized detection by combining CRISPR/Cas12a system with isothermal amplification [24]. A guide RNA was designed specially to the target DNA. Thus, the target DNA worked as an activator to trigger the cleavage of Cas12a enzyme. An unrelated fluorophore quencher (FQ)–labeled reporter was employed for visualized detection. After the RNA binds with the complementary target DNA, the Cas12a enzyme could cleave the FQ-labeled reporter, generating sensitive visualized readout for target amplicons. This study advanced CRISPR/Cas12a system to have a great potential in molecular detection. Apart from the PAM sequence of TTTN, another new PAM (UUUN) was discover by our group, providing a novel strategy for eliminating carryover contamination [25]. Uracil and the uracil-DNA-glycosylase (UDG) enzymes used could help to prevent contamination of DNA amplicons [26].

In this paper, we developed a CRISPR/Cas12a system-based method for PCR by a mini thermal cycler for specific, contamination-free and visualized detection, which could overcome the weakness of other endpoint detection methods mentioned above (Scheme 1). The presented detection method was verified by detecting *V. parahaemolyticus* infected shrimp samples. CRISPR reagent was pre-added inside the lid of the tube and then PCR was carried out on the mini thermal cycler with its cover unclosed. After amplification, the reagents were mixed by centrifugation and incubated immediately at 37 °C for 5 min. CRISPR/Cas12a detection could be performed and the fluorescence readouts could be observed by the naked eye under UV light (254 nm) using a homemade device. The positive samples showed obvious green fluorescence and the negative samples showed no fluorescence.

2. Materials and methods

2.1. Bacterial strains

A total of 25 bacterial strains were used in this study, including *V. parahaemolyticus* isolates, non-*parahaemolyticus Vibrio* isolates and non-*Vibrio* bacteria. Details of bacterial strains can be seen in Table S1, Supporting Information. The strains from American Type Culture Collection (ATCC) were kindly provided by Lin'an Center for Disease Control and Prevention (Lin'an, China).

2.2. NaOH-based DNA crude extraction

200 μL 0.5 M NaOH solution was added into 50 μL of *V. parahaemolyticus* suspension. After intensive mixing and then standing for 3 min at room temperature, the cell lysis solution was diluted (1:50) with Tris-EDTA (TE) buffer. 1 μL of the rude cell lysate was directly used as template for PCR assay. As a comparative test, TIANamp Bacteria DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China) was used for DNA extraction and purification according to its operation manual. DNA templates were dissolved in 100 μL TE buffer and 1 μL DNA was used as template.

2.3. Standard PCR assay

A DNA fragment of *V. parahaemolyticus*-specific thermolabile haemolysin (*tlh)* gene (GenBank No. M36437) was selected as target sequence. The primers employed for PCR amplification were reported in a previous paper by our group [27] and synthesized commercially by Sangon Biotech (Shanghai, China).

Uracil-PCR was carried out in a total 25 μL of reaction mixture containing 0.125 μL TaKaRa Taq HS DNA Polymerase (5 U/μL) (TaKara Biomedical Technology Co., Ltd., Beijing, China), 2.5 μL of 10 × PCR buffer (Mg^{2+} plus) (TaKara Biomedical Technology Co., Ltd., Beijing, China), 0.8 mM dUTPs (0.35 mM dATP, 0.35 mM dUTP, 0.35 mM dGTP, 0.35 mM dCTP) (TaKara Biomedical Technology Co., Ltd., Beijing, China), 0.8 μM of each primer, 4 μM of SYTO 9 dye (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and 1 μL of target DNA. 50 μL mineral oil was added over the reaction mixture to avoid evaporation. The reaction was performed via a Professional Mini 3220 Thermal Cycler (Hangzhou LongGene Scientific Instruments Co., Ltd., China) and the thermal cycle program was 40 cycles of 98 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min.

As a control, Real-time PCR was performed via QuantStudio™3 Real Time PCR System (Thermo Fisher Scientific Inc., Waltham, MA, USA) to collect a fluorescence signal at the end of each cycle. The cycle threshold (Ct) value means the cycle number when the signal in the reaction tube reached the fluorescent threshold. To further validate the specificity of PCR, the melt curve was carried out by gradually increasing the temperature from 60 °C to 95 °C after PCR amplification. The amplicons were also analyzed by 3% agarose gel electrophoresis and then photographed via a ChemiDoc XRS + System (BioRad, Hercules, CA, USA).

2.4. Specificity of PCR detection

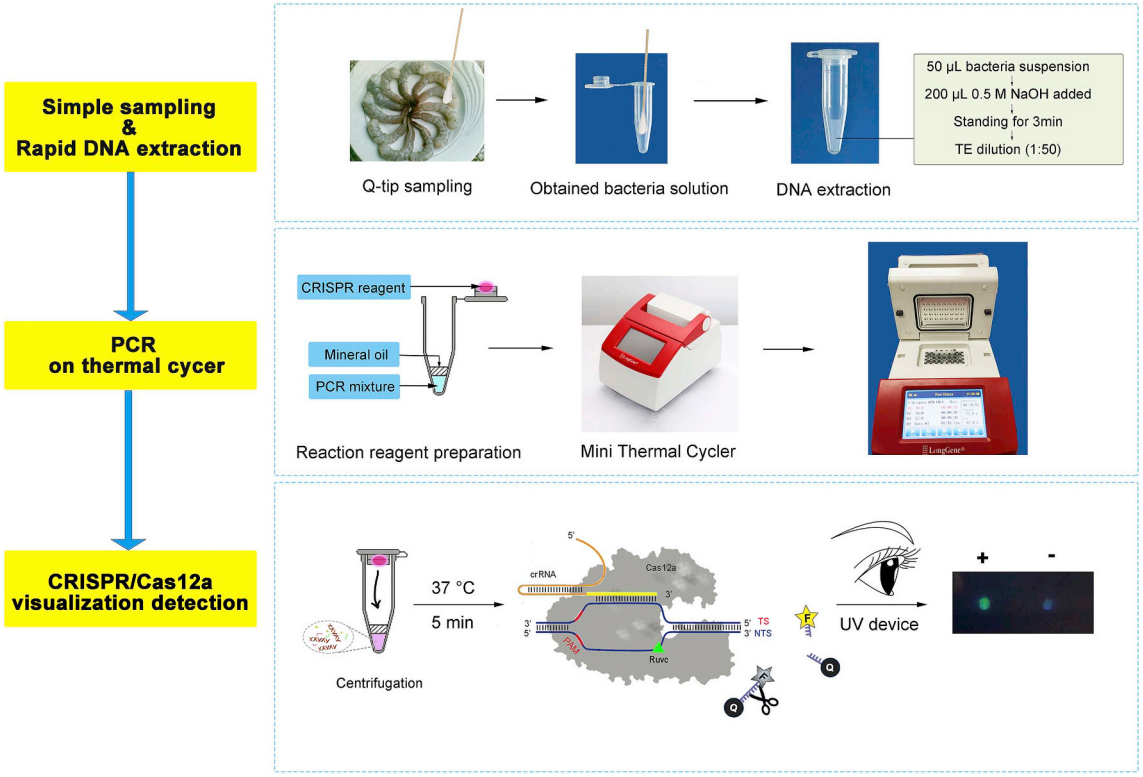
In order to evaluate the specificity of PCR assay, apart from *Vibrio* bacteria, several other main pathogens causing similar clinical symptoms to that by *V. parahaemolyticus*, including *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella typhimurium*, were tested by *tlh*-PCR primers.

2.5. Sensitivity determination of PCR in pure culture

To evaluate the sensitivity of PCR, amplification was performed on a dilution series of DNA extracted from the mixture of *V. parahaemolyticus* strains. The initial concentration of DNA template was 58 ng/μL measured by NanoDrop 2000 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and diluted in 1:10 serials. 1 μL DNA template was added for PCR system and each test was carried out in triplicate.

2.6. Analysis of PCR in spiked shrimp samples

The preparation procedure of spiked shrimp samples was reported by our group previously [27]. Fresh shrimp purchased from Hangzhou local supermarket were de-headed and de-contaminated by placing in a biological safety hood under UV light for 30 min. Then the sterile shrimp samples were incubated respectively in a series of gradient concentrations of *V. parahaemolyticus* suspensions for 30 min under ambient temperature (23 ± 1 °C). Disposable Q-tips were used to wipe the shrimps and collect samples. Then the Q-tips were placed into the 1.5 mL centrifuge tubes and 200 μL of deionized water were added to elute the *V. parahaemolyticus*. DNA was extracted by the NaOH-based method. 1 μL DNA template was added for PCR system and each test was carried out in triplicate.



Scheme 1. Schematic of selective endpoint visualized detection method for *Vibrio parahaemolyticus* based on PCR by using a thermal cycler.

2.7. CRISPR/Cas12a assisted visual detection method

The crRNA for CRISPR detection system was designed specifically for amplification region and synthesized commercially by Sunya Biotech (SunYa Biotechnology Co., Ltd, Hangzhou, China). The sequences of each oligonucleotide used in this work can be found in the Table S2, Supporting Information.

CRISPR reaction was carried out in a total of 20 μL reaction mixture containing 1 × NEB Buffer 2.1 (New England Biolabs Inc., Ipswich, MA, USA), 0.15 μM Cas12a enzyme (New England Biolabs Inc., Ipswich, MA, USA), 0.6 μM crRNA, 2.5 μM probe, 0.4 U recombinant RNase inhibitor (TaKara Biomedical Technology Co., Ltd., Beijing, China), and 2 μL of target amplicon. The reaction mixture was incubated at 37 °C for 5 min. The fluorescence could be detected via a homemade mini-device by our group [25]. A parallel test was performed and the real-time fluorescence was recorded by the QuantStudio™3 Real Time PCR System (Thermo Fisher Scientific Inc., Waltham, MA, USA) as a comparative test.

To avoid uncapping operation, 20 μL of the CRISPR reagent was pre-added inside the tube lid while 25 μL of PCR mixture was added at the bottom of the tube, covered with 50 μL of mineral oil. After PCR amplification, the CRISPR reagent was mixed with the amplicons by centrifugation. Then the CRISPR assisted detection was conducted at 37 °C for 5 min. The visualization results were observed by the naked eye using our own simple observation equipment.

3. Results and discussions

3.1. Design of CRISPR/Cas12a system for tlh-specific PCR

Cas12a enzyme was reported to cleave DNA strands indiscriminately under the guidance of crRNA after binding with the target DNA strands. Therefore, we used CRISPR/Cas12a system as a specific indicator for nucleic acid analysis. As a unique PAM sequence of UUUN was proven to be recognizable by Cas12a enzyme, dUTP was employed as a substitute for dTTP in PCR assay. Then the newly generated dsDNA all contained dUTP. The real-time curve of uracil-PCR was shown to be consistent with standard PCR (Fig. S1, Supporting Information). This allowed amplicons containing uracil to be degraded by UDG enzymes, eliminating the possibility of carryover contamination. In order to achieve selective detection for PCR, we specifically designed a crRNA targeting the amplification sequence. The specific sequence of crRNA could be found in the Table S2, Supporting Information. In the CRISPR/Cas12a system, the target dsDNA, complementary to crRNA, worked as an activator to trigger the cleavage activity of Cas12a-crRNA complex. A universal single-stranded fluorescent probe was employed as the indicator to prove the existence of the target DNA. The CRISPR reaction was carried out at 37 °C for 40 min. Once the cleavage effect of Cas12a enzyme was activated, these fluorescent probes would be cleaved and emit fluorescence. The change in the fluorescence signal could be observed by the naked eye. In addition, comparing to DNA intercalating dyes (e.g., SYBR Green I and SYTO 9) which have a high background signal [10], there is an obvious distinguish between positive and negative samples in CRISPR-based assay.

3.2. Feasibility analysis of CRISPR-PCR on thermal cycler

Commonly, PCR is performed on sophisticated and large-size on-lab instrument since it requires precise temperature changes for thermal cycles. Up to date there are several relative cheaper PCR thermal cyclers commercialized on the market. These could be used for on-site detection or in low-resource areas. Therefore, we hypothesized to employ a thermal cycler to realize on-site application. In this work, the activity of Cas12a enzyme was vital to recognize the target DNA and to conduct visualized detection. The CRISPR reagent, inside the lid of the reaction tube, was separated from the high-temperature zone by a section of the air column and oil phase. This was verified to be feasible in LAMP assay where the reaction temperature is around 65 °C [25]. But thermal cycles in PCR contain a higher temperature over 90 °C, which could probably make Cas12a enzyme inactivated. In order to

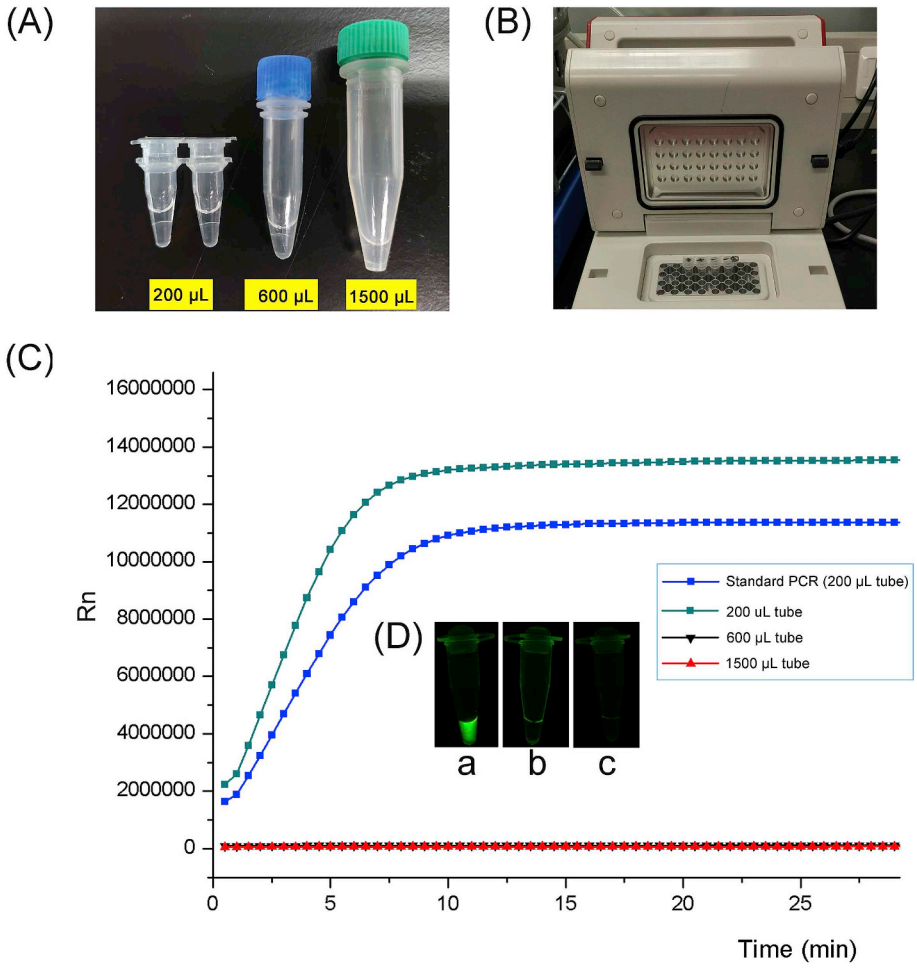


Fig. 1. Feasibility analysis of CRISPR-PCR on thermal cycler. (A) Three types of reaction tubes were used in this study. The volume of the tubes was 200, 600, 1500 µL, respectively. (B) PCR on the thermal cycler. The PCR reaction was carried out on the mini thermal cycler with its cover unclosed. (C) Real-time fluorescence curve of CRISPR detection for PCR products conducted by different tubes. The PCR conducted on the thermal cycler were tested by the three types of tubes. Then 2 µL PCR products were added into 18 µL CRISPR reagent. The results of 200 µL tube (cyan line connected by square symbols) were consistent with those of standard PCR (blue line connected by square symbols) conducted by QuantStudio™3 Real Time PCR System. There is no fluorescence showed from 600 µL tube (black line connected by triangle symbols) and 1500 µL tube (blue line connected by triangle symbols). (D) Visualization of CRISPR detection for PCR products conducted by different tubes. a-c referred to the products from volumes of 200, 600, 1500 µL. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

solve the problem, we did not close the cover of the thermal cycler. Reaction was carried out with half of the reaction tube incubated in the heating block and half bared in the air atmosphere (Fig. 1B). A thick layer of mineral oil is necessary to keep thermal insulation and avoid evaporation. In addition, we hypothesized that large volume of air column would make sense to reserve the activity of enzyme. Thus, in order to verify whether it could conduct PCR, we tested three types of reaction tubes (200, 600, 1500 µL) (Fig. 1A) and found that amplification reaction was successful only in the tube with a volume of 200 µL (Fig. 1C and D). The amplicons were verified by gel electrophoresis (Fig. S2, Supporting Information).

And then the CRISPR/Cas12a assisted visualized detection was tested on the thermal cycler. The CRISPR reagent was pre-added inside the lid of the tube before reaction. After PCR amplification, the CRIPSR reagent was mixed with the products by centrifugation. The visualized detection was carried out at 37 °C for 40 min via QuantStudio™3 Real Time PCR System. High fluorescence was emitted in the positive samples while the negative samples remained no change. The visualized detection results of PCR amplicons from the thermal cycler were consistent with those from QuantStudio™3 Real Time PCR System Fig. S3, Supporting Information). Through the fluorescence signal recorded in real-time, we could confirm that the CRISPR/Cas12a assisted PCR was able to be performed on the thermal cycler with its cover unclosed.

3.3. Specificity and sensitivity of PCR in pure culture

The specificity of primer sets for PCR was evaluated before detection. 25 bacterial strains were analyzed by the Basic Local Alignment Search Tool (BLAST). We found the nucleotide sequences of the primers in this work were specific to *V. parahaemolyticus* because only five isolates of *V. parahaemolyticus* showed positive results while other 10 *Vibrio* spp. and 10 non-*Vibrio* isolates showed negative results. But when carrying out standard PCR using SYTO 9 dye, not only *V. parahaemolyticus* isolates but *Salmonella typhimurium* isolate showed positive results (Fig. 2A). As can be seen from the results of gel electrophoresis (Fig. 2B), a fragment of 150–200 bp length was amplified in *Salmonella typhimurium* isolate (Lane 8). It was distinguishing from the target amplicons of which the length was 354bp. As no band was shown in blank control which used RNase-free water as a template, line 8 referred to non-specific amplification. The sequence of non-specific amplicons was tested by gene sequencing and could be seen in Table S2, Supporting Information. Although it is a common solution by optimizing the reaction system or redesigning the primers, it is time-consuming, labor-intense and might not be effective to eliminate non-specific amplicons or primer dimer. In this way, detection assays that are not related to specific sequences have some drawbacks because false positive results could occur. CRISPR/Cas12a system could be employed for selective nucleic acid analysis. 2 µL of products from PCR was added into the 18 µL CRISPR reagent. The mixture was performed at 37 °C for 40 min. As shown in Fig. 2C, only 3 *V. parahaemolyticus* isolates generated green fluorescence that was easily observed by the naked eye while no positive result was observed in other samples. This indicated that CRISPR/Cas12a assisted visualized detection assay had a high level of specificity. It contributes a lot for practical application, especially for on-site detection.

To evaluate the sensitivity of CRISPR/Cas12a assisted PCR, a series of 10-fold gradient dilutions of *V. parahaemolyticus* genomic DNA was used as templates for PCR amplification. The initial concentration of

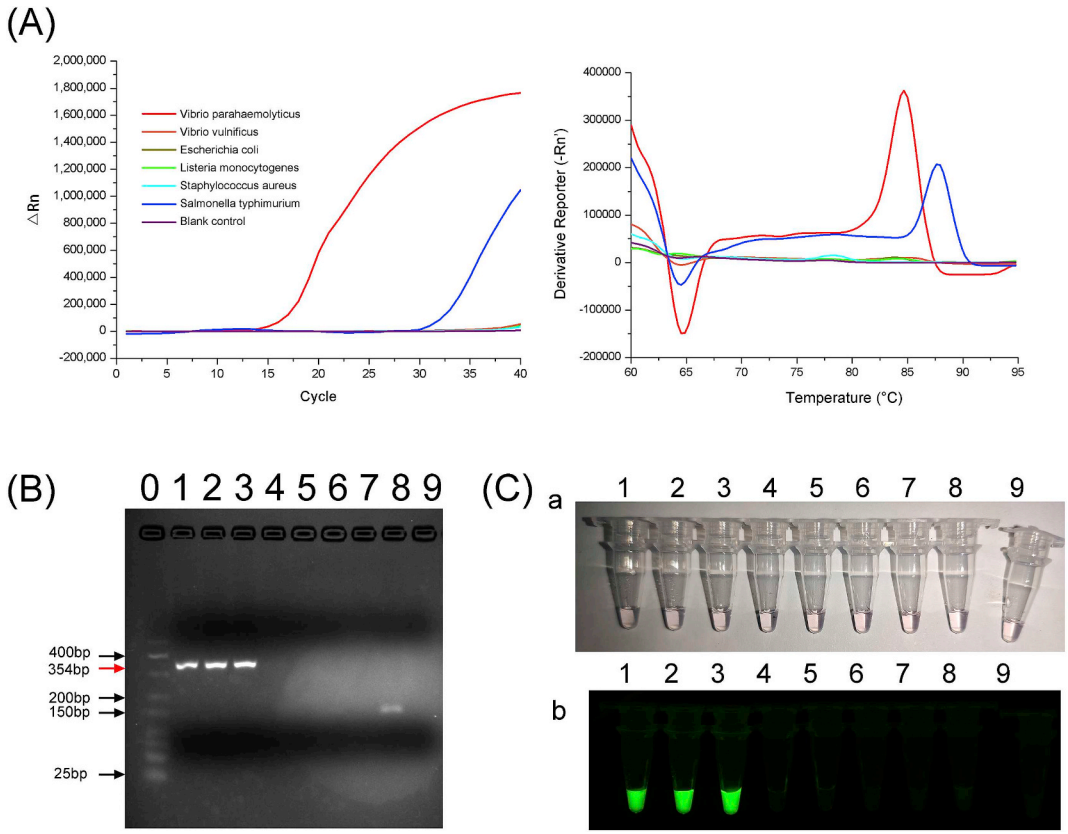


Fig. 2. Specificity assessment of CRISPR/Cas12a assisted PCR for bacteria detection. (A) Real-time fluorescence and melt curve of standard PCR. (B) Visualization of amplicons by agarose gel electrophoresis. Lane 1–3, positive samples of 3 *Vibrio parahaemolyticus* isolates. Lane 4–8, negative samples of *Vibrio vulnificus*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella typhimurium*, respectively. Lane 9, RNase-free water as blank control. (C) Visualization of CRISPR detection. 2 μL PCR amplicons were added into 18 μL CRISPR reagent. (a) In visible light (b) under UV light.

template DNA amount was 58 ng/μL, equivalent to 1.02×10^7 copies of genomic DNA per reaction (calculated based on the estimated complete genome size of *V. parahaemolyticus* of 5.17 Mbp [28]). Results showed that a band of 354 bp length was observed at concentrations ranging from 1.02×10^7 to 1.02×10^2 copies/μL, indicating the limit of detection (LOD) of PCR was 1.02×10^2 copies/μL (Fig. 3B). Standard PCR utilizing SYTO 9 was conducted via QuantStudio™3 Real Time PCR System as a contrast (Fig. 3A). The results were consistent. Then the amplicons were detected by CRISPR system. In order to avoid uncapping operation, 20 μL of CRISPR reagent was pre-added into the lid of tube when 25 μL PCR reagent was added at the bottom of the tube with a layer of 50 μL mineral oil before amplification. Then PCR was carried out on the thermal cycler with the cover unclosed. After amplification, the CRISPR reagent was mixed with the PCR products by centrifugation. Then the tube was incubated at 37 °C for 40 min immediately. The sensitivity of CRISPR/Cas12a assisted PCR was assessed on the thermal cycler and the end-point image of the sensitivity assessment was shown in Fig. 3C. There was no apparent distinction of the brightness between the positive tubes at gradient dilution concentrations. This might due to that each of the activated Cas12a enzymes could cleavage a large number of fluorescent probes once the amplicons were recognized and complementarily paired with the crRNA. The fluorescence signal was determined by the number of fluorescent probes cut. To shorten the time of visualized detection, the mixture of CRISPR reagent and PCR amplicons was incubated via the homemade device. The real-time fluorescence signal of positive and negative samples was observed by the naked eye. We found that the positive sample at the lowest detectable concentration could show high fluorescence in 5 min (Video S1, Supporting Information). Therefore, PCR following with a 5 min CRISPR/Cas12a assisted visual detection was sufficient in this work.

(Supplementary data related to this article can be found at https://doi.org/10.1016/j.talanta.2020.120818.

A video recording the real-time fluorescence of CRISPR visualized detection Video S1) (MP4).

3.4. Analysis of CRISPR- PCR in spiked shrimp samples

Then, we considered employing the CRISPR assisted PCR to detect real samples for assessing its practical application. The assay of preparation of spiked shrimp samples was described in our previous work [29]. A series of gradient concentrations of *V. parahaemolyticus* (1.02×10^6, 1.30×10^5, 4.50×10^4, 3.00×10^3, 2.50×10^2, 0 CFU/g) infected shrimp samples was prepared. Disposable Q-tips were used to wipe the shrimp and collect samples, simplifying the operation of sample treatment. DNA was extracted by the NaOH-based method and 50-fold diluted with TE buffer. After mixing CRISPR reagent with amplicons, the fluorescence was detected by the homemade device. A parallel test was carried out via QuantStudio™3 Real Time PCR System to compare its intensity of fluorescence. Results showed that the LOD of detection from shrimp samples ranged from 10^2 to 10^6 CFU/g. Fig. 4A showed that after 5 min, although the intensity of fluorescence is gradient, there is little difference in observation by the naked eye. In addition, after a long time incubation with CRISPR, different gradient concentrations of *V. parahaemolyticus* infected shrimp samples reached a similar intensity of fluorescence (Fig. 4B), indicating this method is sensitive. Consequently, the presented method showed great feasibility for detection of *V. parahaemolyticus* from spiked shrimp samples.

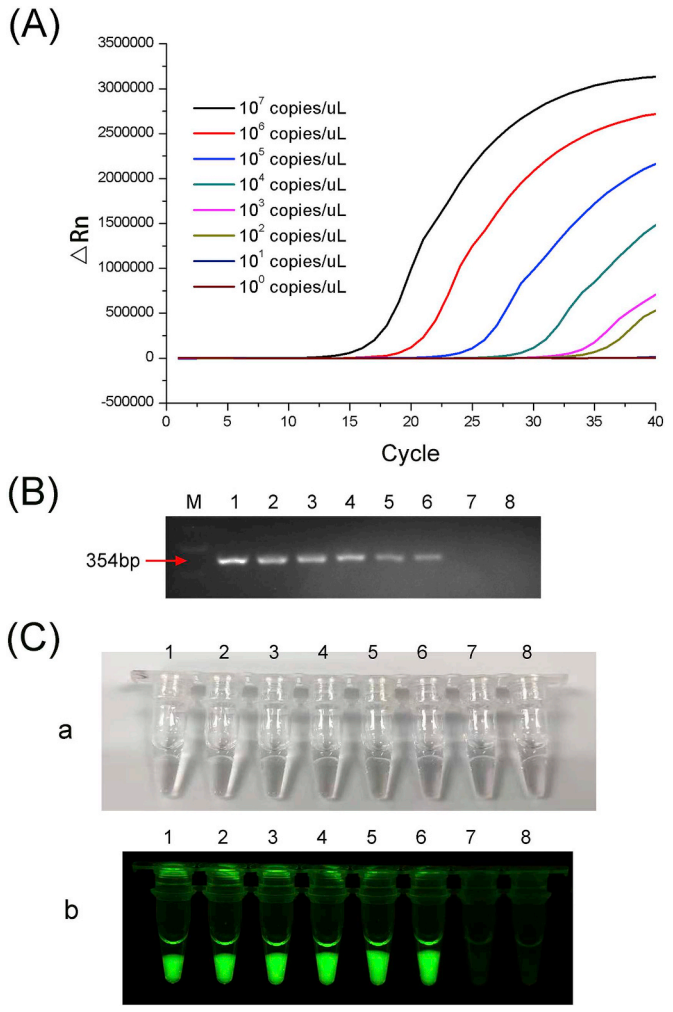


Fig. 3. Sensitivity assessment of CRISPR/Cas12a assisted PCR for the mixture of 3 *V. parahaemolyticus* strains. Lane 1–7, 10-fold gradient dilutions of DNA template from 1.02×10^7 to 1.02×10^1 copies/μL. Lane 8, RNase-free water as blank control. (A) Real-time PCR. (B) Visualization of PCR products by agarose gel electrophoresis. (C) Visualization of CRISPR/Cas12a assisted PCR. (a) In visible light (b) under UV light. The CRISPR reagent was pre-added into the lid before reaction. After amplification, rapid centrifugation to mix the reagents for selective visual detection.

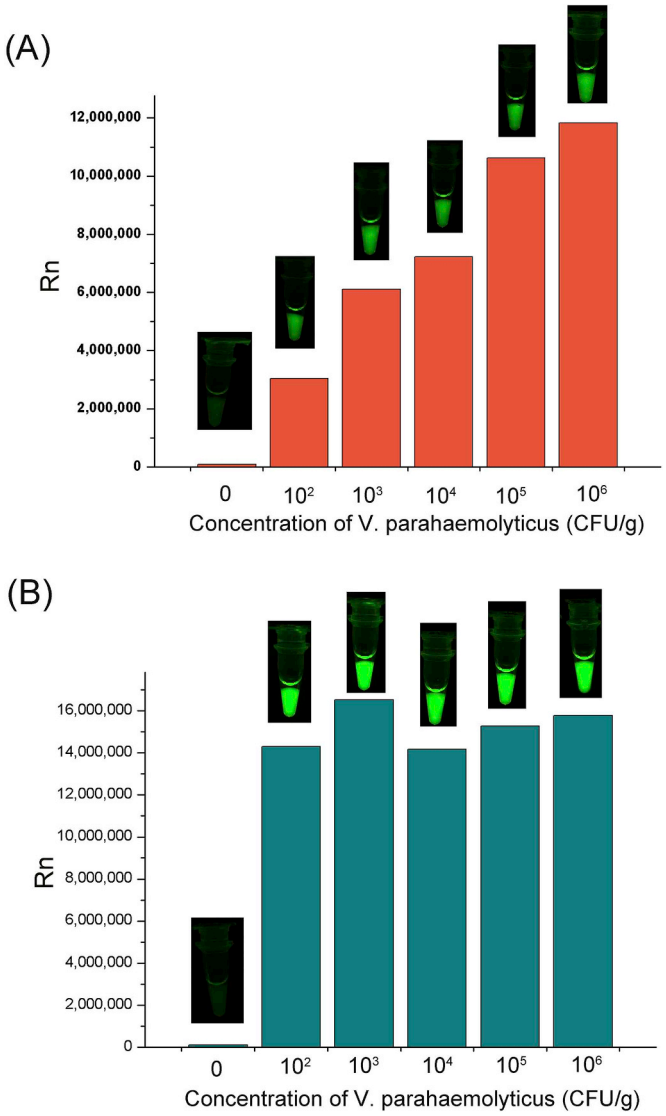


Fig. 4. Analysis of CRISPR-PCR in spiked shrimp samples. After amplification, the CRISPR reagent was mixed with the PCR amplicons and incubated in 37 °C by the homemade device. The image of fluorescence was observed by the naked eye and photographed. As a contrast, a parallel test was carried out and the real-time fluorescence was recorded by the QuantStudio™3 Real Time PCR System. (A) Incubating for 5 min (B) incubating for 40 min. In real shrimp samples, the LOD was 10^2 CFU/g. For the sample without target DNA, there was no fluorescence.

4. Conclusions

In this paper, we developed a novel, specific and practical PCR method for visualized endpoint detection of *Vibrio parahaemolyticus* using a mini thermal cycler. Compared to standard PCR, this developed method employed CRISPR/Cas12a system and uracil to achieve high specificity and avoid carryover contamination. This greatly reduced the possibility of false positive results. In addition, the use of a mini thermal cycler reduced the need of bulky, expensive instrument and advanced the possibility of on-site application of PCR. As PCR is still the mainstream of molecular diagnosis, this developed method is not only applicable for detection of pathogens but also has potential in many other fields, from food safety analysis to medical diagnosis.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgement

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Abbreviations

PCR	Polymerase Chain Reaction
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
dsDNA	Double-stranded DNA
crRNA	CRISPR RNA
PAM	Protospacer-adjacent Motif

ssDNA	Single-stranded DNA
UDG	Uracil-DNA-glycosylase
ATCC	American Type Culture Collection
TE	Tris-EDTA
tlh	thermolabile haemolysin
Ct	Cycle Threshold
BLAST	Basic Local Alignment Search Tool
LOD	Limit of Detection

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2020.120818.

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