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ORIGINAL PAPER

Development and Evaluation of a Novel Armored RNA Technology Using Bacteriophage Qβ

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Abstract

Foodborne viruses are a global threat to food safety. Real-time reverse transcription polymerase chain reaction (RT-PCR) is the most commonly used method to detect viral RNA in food. Armored RNA (AR) prepared using the MS2 phage system is a successful positive control for detecting foodborne viruses and is an important quality control process when using real-time RT-PCR. In this study, we report a novel technology for preparing AR using bacteriophage Qβ and compare its stability with AR prepared using the MS2 phage system for packaging norovirus detection target RNA. AR could be successfully and efficiently produced using the developed bacteriophage Qβ system. Two types of AR–AR-QNoV prepared using the Qβ system and AR-MNoV prepared using the MS2 system—were stored at different temperatures for different durations. After incubating at − 20 °C for 360 days, the copy numbers of AR-QNoV and AR-MNoV decreased by 8.9% and 35.9%, respectively. After incubating at 4 °C for 60 days, the copy numbers of AR-QNoV and AR-MNoV decreased by 12.0% and 38.9%, respectively. After incubating at 45 °C, the copy numbers of AR-QNoV decreased by 71.8% after 5 days, whereas those of AR-MNoV decreased by 92.9% after only 4 days. After 5 days, AR-MNoV could not be detected using real-time RT-PCR. There was a significant difference in copy numbers decrease rate between AR-QNoV and AR-MNoV at three different temperatures ($P < 0.05$). Therefore, AR prepared using the new bacteriophage Qβ system is more stable than the traditional AR, making the developed strategy a good candidate for AR preparation and quality control.

Keywords Armored RNA · Qβ phage · Stability · Comparison · Norovirus

Introduction

In spite of food being safer at present than ever before, foodborne diseases remain a major cause of worldwide morbidity and mortality. However, the actual global consumption of unsafe food is difficult to determine (Bosch et al. 2016). Based on the incidence of reported foodborne diseases and their severity (including mortality), human enteric viruses have been increasingly recognized as important causes of infections worldwide (Koopmans et al. 2002) because of their frequent transmission through food. Human noroviruses, hepatitis A virus, human rotavirus, and hepatitis E virus are the most frequent causes of foodborne infections (Koopmans et al. 2002; Bosch et al. 2016).

Identifying such viruses in food samples is technically challenging because of the low genomic copy numbers (10^2–10^6) or virus particles per gram of the contaminated food samples (Polo et al. 2015) as well as the effects of complex food sample matrices and inhibitors (Malorny et al. 2003; Mikel et al. 2016; Niesters 2002; Stals et al. 2012). Therefore, sensitive, specific, rapid, high-throughput, and reliable molecular assays, such as reverse transcription polymerase chain reaction (RT-PCR) or real-time RT-PCR, have been widely adopted in the surveillance of foodborne viruses (Zhang et al. 2015); however, potential for false-negative results remains a concern with any molecular assay. Several factors, such as the presence of RT-PCR inhibitors, poor target RNA recovery during extraction, degradation of target RNA before amplification, errors in setting up a reaction, and use of a degraded reagent, can lead to false-negative results (Akane et al. 1994; Khan et al. 1991; Lantz et al. 1997; Monteiro et al. 1997; Wilson 1997). Therefore, the

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presence of a positive control or reference material is useful to monitor molecular assay procedures and to ensure their accuracy (Das et al. 2006).

Several biological materials, such as live or inactivated viruses, DNA, and naked RNA, have been used as controls for molecular diagnostic assays of RNA viruses (Aarthi et al. 2004; Pisani et al. 2008; Song et al. 2011); however, each has some inherent disadvantages, such as the biosecurity risk of live viruses, risk of residual activity of inactivated viruses, inability to evaluate the processes of reverse transcription and RNA extraction, and relative instability of naked RNA.

The development of armored RNA (AR) using the MS2 phage packaging system has overcome the abovementioned disadvantages (Pasloske et al. 1998). With increased safety, stability, and accuracy, AR has been widely used in the diagnostic assays of RNA viruses (Beld et al. 2004; Bressler and Nolte 2004; Eisler et al. 2004; Karatayli et al. 2014; Zhao et al. 2007); however, because of high cost and time required for periodic monitoring and evaluation, a better positive control or reference material, such as AR with better stability, is required.

The stability of bacteriophage Qβ (*Leviviridae*: *Allolevivirus*) (Ashcroft et al. 2005; Bundy and Swartz 2011) makes it an attractive candidate for virus-like particle (VLP)-based applications. In this article, we describe a novel technology for preparing AR using the Qβ packaging system. In addition, we demonstrate the packaging of a target human norovirus RNA (genogroup II, GII) using the developed Qβ system and compare its stability with the traditional AR prepared using the MS2 system.

Materials and Methods

Construction of Recombinant Plasmid pET-QINoVGII

According to the bacteriophage Qβ genome (GenBank AB971354, 61–2367 nt) and norovirus (GII) detection target sequence (GenBank x86557, 5012–5100 nt) (Kageyama et al. 2003; Loisy et al. 2005), QINoVGII, a nucleic acid fragment, was synthesized by BGI Tech Solutions Beijing LiuHe Co., Ltd. From the 5′ end to the 3′ end, QINoVGII contains an upstream homologous sequence (5′-CAGCAA ATGGGTCGC-3′) of the prokaryotic expression vector pET-28a(+) (Novagen®, Germany); Qβ maturase-encoding gene, capsid protein-encoding gene; packaging site sequence; cDNA corresponding to norovirus (GII) detection target RNA; multiple cloning sites (*Apa*I, *Kpn*I, *Pst*I, *Spe*I, *Sph*I, and *Not*I); and a downstream homologous sequence (5′-GTG GTGGTGGTGGTG-3′) of pET-28a(+) (Fig. 1). QINoVGII was subcloned into pET-28a(+) using recombinases. Briefly, pET-28a(+) was double digested using restriction enzymes *Bam*HI and *Xho*I (TaKaRa Bio, Inc., Shiga, Japan). The

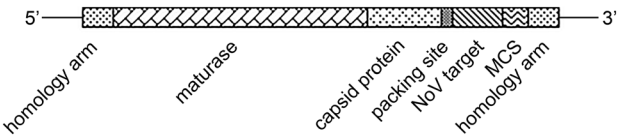

Fig. 1 Schematic of the structure of DNA fragment QINoVGII

reaction mixtures contained 2.0 µL (~ 800 ng) plasmid pET-28a(+), 1.5 µL (15 U/µL) *Bam*HI, 1.5 µL (10 U/µL) *Xho*I, 3.0 µL 10 × K buffer, and 22.0 µL ddH_2O. pET-28a(+) was digested at 37 °C for 2 h and then purified using the TaKaRa MiniBEST Agarose Gel DNA Extraction Kit (TaKaRa) according to the manufacturer's instructions. QINoVGII was then directly cloned into linearized pET-28a(+) using the ClonExpress®II One Step Cloning Kit (Vazyme Biotech Co., Ltd.) according to the manufacturer's instructions. The reaction system and conditions were as follows: 1.0 µL (~ 50 ng/µL) linearized pET-28a(+), 1.0 µL (~ 20 ng/µL) QINoVGII, 4.0 µL 5 × CE II buffer, 2.0 µL Exnase®II, and 12.0 µL ddH_2O. The mixture was incubated at 37 °C for 30 min and then transformed into *Escherichia coli* Top10 competent cells [TIANGEN Biotech (Beijing) Co., Ltd., China]. The positive recombinant plasmid—pET-QINoVGII—was confirmed by restriction digestion and sequenced by BGI Tech Solutions Beijing LiuHe Co., Limited. The entire procedure of AR development using bacteriophage Qβ is presented in Fig. 2.

pET-QINoVGII Expression

pET-QINoVGII was expressed in *E. coli* as described previously (Sambrook et al. 1989). Briefly, pET-QINoVGII was transformed into *E. coli* BL21 (DE3) competent cells (TIANGEN). The recombinant *E. coli* harboring plasmid pET-QINoVGII was cultured overnight at 37 °C in lysogeny broth (LB) medium supplemented with 50 µg/mL kanamycin. The suspension was diluted 1:100 using fresh LB medium and incubated at 37 °C while shaking at 200 rpm for 3–4 h. When the cells reached an optical density at 600 nm $(OD)_{600}$ of 0.6, 0.8 mM isopropyl-β-D-thiogalactopyranoside (IPTG) was added to continue induction for 12 h. The expression product—VLPs of bacteriophage Qβ containing the target RNA of norovirus (AR-QNoV)—was analyzed using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Production and Purification of Armored RNA

After confirmation using SDS-PAGE, recombinant *E. coli* suspension was diluted and cultured in 200 mL LB medium as described earlier. The cells were harvested by centrifuging at 10,000 rpm for 10 min at 4 °C, and the pellet was

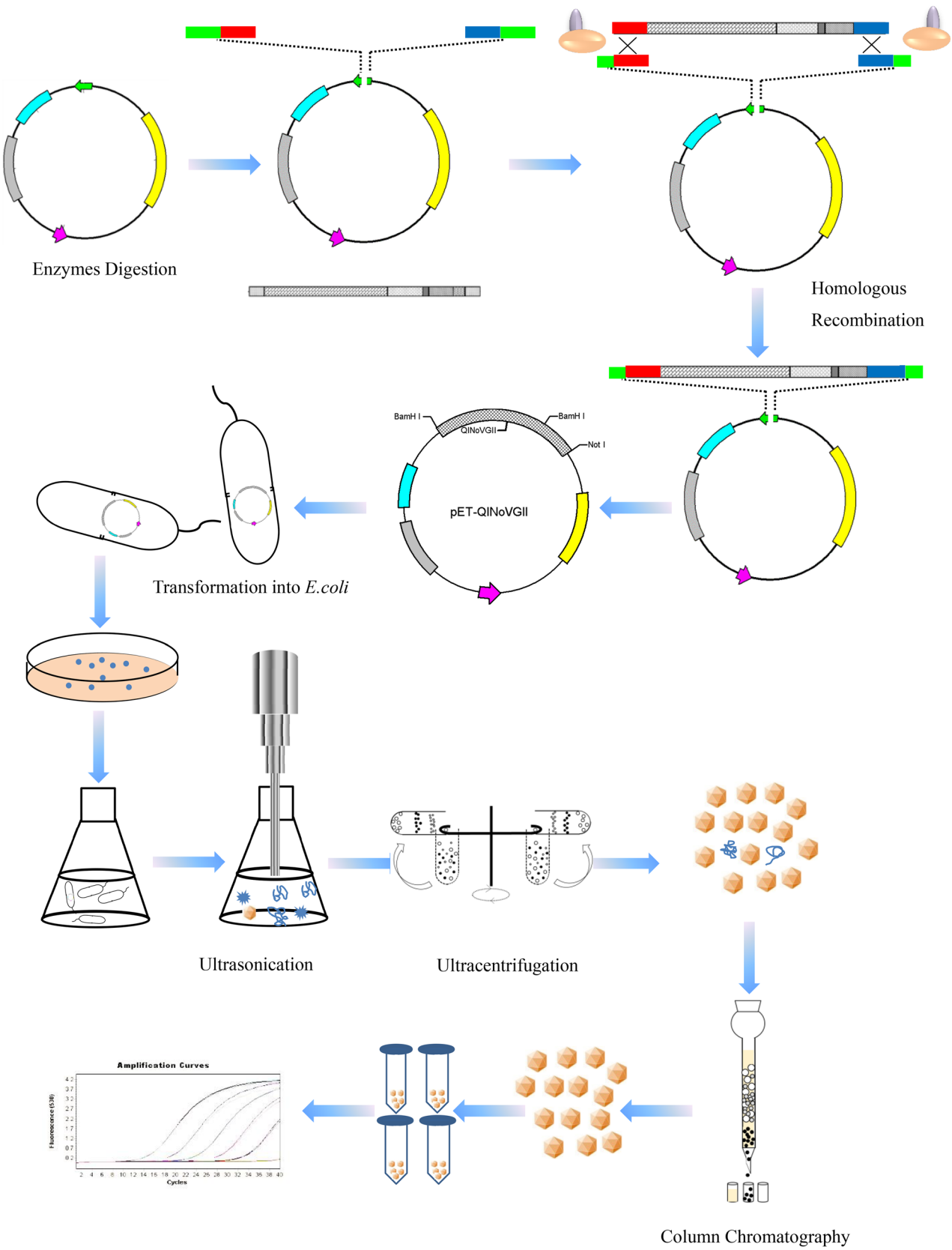

Fig. 2 Schematic of the development and purification of armored RNA using the bacteriophage Qβ system

resuspended in a sonication buffer (2 mM ethylenediaminetetraacetic acid, 100 mM NaCl, 50 mM Tris–HCl, pH 8.0, 0.1% Triton X-100) and lysed using an ultrasonic homogenizer JY92-IIN (Ningbo Scientz Biotechnology Co., Ltd.). After centrifuging at 10,000 rpm for 20 min at 4 °C, the supernatant was incubated with 100 U DNase I and 200 U RNase A at 37 °C for 2 h. The AR-QNoV particles in the bacterial lysate were further purified using CsCl density gradient ultracentrifugation at 80,000 rpm for 5 h at 4 °C (CP100WX, Hitachi, Tokyo, Japan). The main fractions were pooled and purified by dialysis with phosphate-buffered saline (pH 7.2) against sonication buffer. AR-QNoV was further purified using Sephacryl S-200 gel exclusion chromatography (BioLogic DuoFlow chromatography system, Bio-Rad Laboratories, USA). The absorbance of purification products was measured at 260 (A_{260}) and 280 (A_{280}) nm, and the products were separated and identified using SDS-PAGE.

Transmission Electron Microscopy

AR-QNoV was negative stained with 2% phosphotungstic acid and its morphology was observed using a transmission electron microscope (TEM; JEM-1200EX, JEOL Ltd., Japan) operated at 100 kV.

Residual Plasmid DNA Detection

The purity of AR-QNoV of each stock solution was tested using real-time PCR (without RT) of the cloned cDNA of the norovirus (GII) detection target RNA sequence with the primers QNIF2d/COG2R and probe QNIFS (250 nM) (Kageyama et al. 2003; Loisy et al. 2005). The sequences were as follows: QNIF2d: 5′-ATGTTCAGRTGGATGAGR TTCTCWGA-3′, COG2R: 5′-TCGACGCCATCTTCATTC ACA-3′, QNIFS (Probe): 5′-FAM-AGCACGTGGGAGGGC GATCG-TAMARA-3′.

AR-QNoV was added to the PCR mixture as a template. Each 20-μL PCR mixture contained 10.0 μL 2 × *Premix Ex Taq* (probe qPCR) (TaKaRa), 0.4 μL (10 μM) QNIF2d, 0.4 μL (10 μM) COG2R, 0.8 μL (10 μM) QNIFS, 6.4 μL RNase-free ddH_2O, and 2.0 μL AR-QNoV. PCR was performed (Roche LightCycler 480 System, Roche, Switzerland) as follows: 1 cycle at 95 °C for 10 s, followed by 40 cycles of denaturing at 95 °C for 5 s and annealing at 60 °C for 20 s. Samples were assayed in triplicates using positive (pET-QINoVGII as template) and blank (RNase-free ddH_2O as template) controls in each assay.

AR-QNoV Quantification

The components obtained via AR development and purification were considered the AR-QNoV original solution, which was diluted 100 times using RNase-free ddH_2O and used as the AR-QNoV stock solution. The stock solution was further diluted 1000 times with RNase-free ddH_2O and used as the AR-QNoV working solution, which was stored in RNase-free Eppendorf tubes until further use.

The AR-QNoV working solution was quantified as described previously (Sun et al. 2013; Monjure et al. 2014) with slight modifications. Briefly, RNA was extracted from the stock solution using TRIzol™ (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and quantified using the absorbance-based nucleic acid quantification method with a NanoPhotometer (Implen Gmbh, München, Germany). The concentration of the AR-QNoV stock solution was analyzed using the following formula: $(6.02 \times 10^{23}$ copies/mol) × RNA concentration (ng/μL) × (10^{-9})/(nucleotide number packed in AR × 340) = copies/μL.

10-fold serial dilutions of RNA were then used as external standards to quantify the concentration of the AR-QNoV working solution.

Real-time RT-PCR was performed using the One Step Primescript™ RT-PCR Kit (TaKaRa); 20-μL PCR mixture contained 10 μL 2 × one step RT-PCR Buffer III, 0.4 μL (5 U/μL) *TaKaRa Ex Taq* HS, 0.4 μL PrimeScript RT Enzyme Mix II, 0.3 μL QNIF2d, 0.3 μL COG2R, 0.4 μL QNIFS, 2.0 μL template RNA (10-fold serial diluted RNA or RNA extract from the working solution), and 6.2 μL RNase-free ddH_2O. Real-time RT-PCR (LightCycler 480 System) was conducted as follows: 1 cycle at 42 °C for 5 min, followed by 1 cycle at 95 °C for 10 s, 40 cycles of denaturing at 95 °C for 5 s, and annealing at 60 °C for 20 s. Fluorescent signals were assessed during the annealing step.

Exogenous calibration curves were generated by plotting the cycle threshold (C_t) values against log-transformed concentrations of 10-fold (log) serial dilutions of RNA extracted from the AR-QNoV stock solution. The copy numbers of AR-QNoV in the working solution were determined by plotting C_t values obtained from 15 tests against the exogenous calibration curves.

AR Preparation Using the MS2 Phage System

As a parallel study for stability, AR based on the MS2 phage (Genbank V00642) containing the GII norovirus detection target (AR-MNoV) was prepared, purified, and quantified using the same strategy mentioned above.

Stability Test

For stability test, the AR-QNoV and AR-MNoV working solutions were further diluted 100 times using RNase-free ddH_2O and 100-μL aliquots were made into several hundred RNase-free Eppendorf tubes; this volume was sufficient for TRIzol extraction. The samples were individually incubated

at 45 °C, 4 °C, and − 20 °C and assessed in triplicates at different temperatures and durations as follows: − 20 °C (15, 30, 60, 90, 180, 270, and 360 days), 4 °C (5, 10, 20, 30, 40, 50, and 60 days), and 45 °C (1, 2, 3, 4, 5, 6, and 7 days); samples were stored at − 80 °C until the completion of experiments. Finally, all samples were processed and quantified in triplicates in a single run using the real-time RT-PCR protocol mentioned in section "AR-QNoV quantification". Samples obtained at each temperature/time point were assayed in triplicates and averaged.

Statistical Analyses

Student's *t* test was performed to calculate the difference between AR-QNoV and AR-MNoV at different temperatures. $P < 0.05$ was considered significant. All statistical analyses were performed using GraphPad Prism v. 6.01 (GraphPad Software, San Diego, California, USA; www.graphpad.com).

Results

Construction of Recombinant Plasmid pET-QINoVGII

The recombinant plasmid pET-QINoVGII was confirmed using restriction digestion, and the digested DNA products were separated using 1.0% agarose gel electrophoresis and visualized under ultraviolet light. A single band (~ 7.8 kb) with *Not*I and three bands (~ 5.3 + ~ 1.8 + ~ 0.7 kb) with *Not*I/*Bam*HI were obtained (Fig. 3). Subsequent sequencing verification (data not shown) confirmed that pET-QINoVGII was successfully constructed.

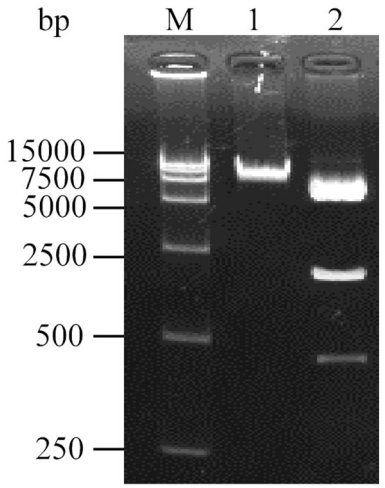

Fig. 3 Identification of the recombinant plasmid pET-QINoVGII using agarose gel electrophoresis. *Lane M* DNA molecular weight marker. *Lane 1* pET-QINoVGII digested with *Not*I. *Lane 2* pET-QINoVGII digested with *Not*I and *Bam*HI

pET-QINoVGII Expression

pET-QINoVGII expression in *E. coli* BL21 was analyzed using SDS-PAGE. pET-QINoVGII expression was detected at ~ 14 kDa (Fig. 4, lane 3), which is close to the theoretical size of the bacteriophage Qβ capsid protein, thereby demonstrating the successful expression of pET-QINoVGII in *E. coli* BL21. In addition, pET-MINoVGII expression in *E. coli* BL21 was confirmed using SDS-PAGE (Fig. 4, lane 4), which showed that the molecular weight of the product was close to that of the MS2 phage capsid protein (13.8 kDa). Although the molecular weight of the capsid protein was slightly different between the two phage systems, SDS-PAGE could not definitively distinguish these because of its low resolution.

Purification of AR

AR was purified using CsCl density gradient ultracentrifugation and Sephacryl S-200 exclusion chromatography. A_{260} and A_{280} of the different sample aliquots were measured using a spectrophotometer; both absorbency peaks were detected in aliquot 22 (Fig. 5), suggesting the presence of the most pure AR. Purified AR was analyzed again using SDS-PAGE, which showed a satisfactory single and clear band at the correct placement (Fig. 6).

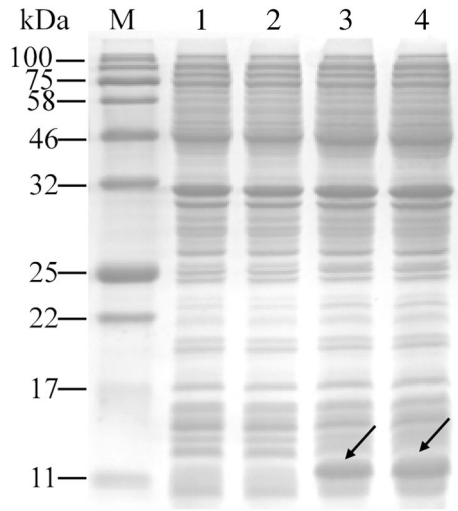

Fig. 4 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of recombinant Qβ phage protein coat expression in *Escherichia coli* BL21. *Lane M* protein molecular weight marker. *Lane 1* proteins of *E. coli* BL21. *Lane 2* proteins of *E. coli* BL21 harboring pET-28a(+). *Lane 3* proteins of *E. coli* BL21 harboring pET-QINoVGII. *Lane 4* proteins of *E. coli* BL21 harboring pET-MINoVGII. Arrow indicates the recombinant protein

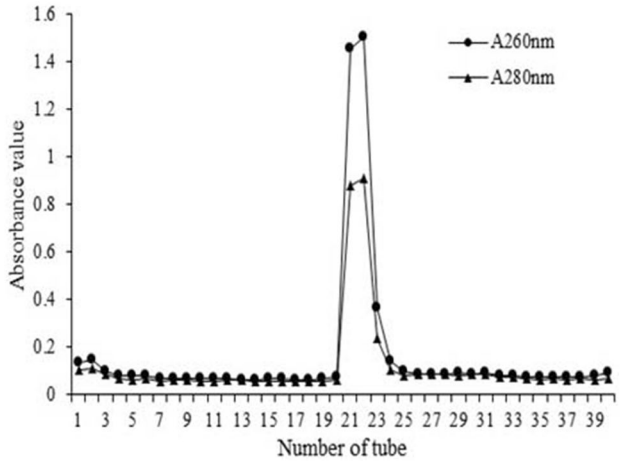

Fig. 5 Absorbance analysis of AR-QNoV purified by gel exclusion chromatography

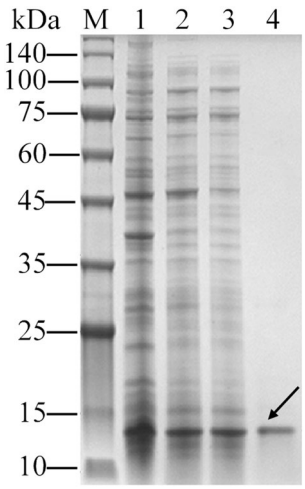

Fig. 6 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of recombinant Qβ phage protein coat purification expressed in *E. coli* BL21. *Lane M* protein molecular weight marker. *Lane 1* whole proteins of *E. coli* BL21 harboring pET-QINoVGII. *Lanes 2–3* virus-like particles (VLPs) purified using CsCl gradient ultracentrifugation alone. *Lane 4* VLPs purified using CsCl gradient ultracentrifugation combine with column chromatography. Arrow indicates the purified protein

TEM

Many VLPs could be observed using TEM (Fig. 7). The VLP structure was complete, with a uniform size and a diameter (~ 28 nm), which make them indistinguishable in morphology from native bacteriophage Qβ (Vasiljeva et al. 1998). Thus, the Qβ phage capsid protein expressed in *E. coli* BL21 could efficiently self-assemble into VLPs.

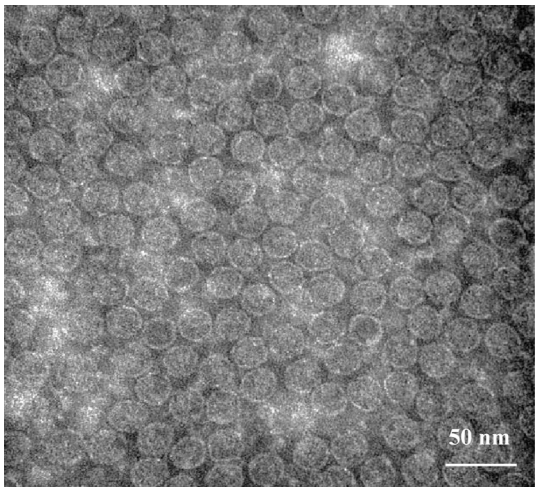

Fig. 7 Transmission electron microscopy (TEM) of purified AR-QNoV. Scale = 50 nm

Residual Plasmid DNA Detection

Real-time PCR was conducted to detect any recombinant plasmids in AR-QNoV purified by gel chromatography. The purified AR-QNoV in the working or stock solution, produced negligible amplification signals even in large amounts similar to the negative control (ddH_2O). Meanwhile, the positive control (recombinant plasmid pET-QINoVGII) produced significant amplification signals. These results indicated that there were no plasmid residues in purified AR-QNoV.

AR-QNoV Quantification

Based on sample serial-dilution gradients and C_t values, the standard curve was derived as $y = -3.53x + 46.84$ ($R^2 = 0.9914$, efficiency 0.9199). The target RNA concentration in AR-QNoV working solution was 5.3×10^{10} copies/μL. After diluting with RNase-free ddH_2O, several hundreds of AR-QNoV stock solutions (100 μL, 5.3×10^8 copies/μL) were prepared for subsequent stability research. AR-MNoV was prepared, quantified, diluted, and separated at a concentration of 3.3×10^8 copies/μL using the same method (data not shown).

The concentration of AR-QNoV preparing using bacteriophage Qβ was remarkably high, and a single lot of AR-QNoV from 0.5 L *E. coli* cells could generate up to 10^{17} copies of norovirus target RNA, which is approximately 100 times higher than the number of copies produced by AR prepared using the MS2 packaging system (Pasloske et al. 1998). The production efficiency of the Qβ packaging system appeared to be higher than that of MS2, indicating that the developed AR preparation using bacteriophage Qβ yielded adequate AR for practical applications.

Stability Tests

The stability of two types of ARs—AR-QNoV and AR-MNoV—incubated at different temperatures and for different durations was analyzed and compared. After incubation at − 20 °C for 360 days, the copy numbers of AR-QNoV and AR-MNoV decreased by 8.9% and 35.9%, respectively (Fig. 8a). After incubating at 4 °C for 60 days, the copy numbers of AR-QNoV and AR-MNoV decreased by 12.0% and 38.9%, respectively (Fig. 8b). After incubating at 45 °C, the copy number of AR-QNoV decreased by 71.8% after for 5 days and that of AR-MNoV decreased by 92.9% after only 4 days. At 5 days, AR-MNoV could not be detected using real-time RT-PCR (Fig. 8c). Thus, there was a significant difference in copy numbers between AR-QNoV and AR-MNoV at different temperatures ($P < 0.05$). Therefore, AR prepared using bacteriophage Qβ was better than AR prepared using the MS2 system at commonly used temperatures of AR storage, transportation, or encounters.

Discussion

Cloning of a target DNA fragment into a plasmid vector is a common molecular strategy, however, because the genes encoding Qβ maturase and capsid protein and cDNA corresponding to the GII norovirus detection target contain nearly all restriction enzyme sites at the multiple cloning sites of pET-28a(+), the method of "enzyme digestion + DNA ligase", could not be used in this study. Therefore, we used recombinases to insert the QINoVGII fragment containing the homologous sequence into linearized pET-28a(+) plasmid without considering various enzyme restriction sites. In addition, we constructed several restriction enzyme sites, such as those for *Apa*I and *Kpn*I, at the 3′ end of QINoVGII, which were not present in the rest of the QINoVGII fragment. These sites further enabled the insertion of other target cDNA fragments from the viruses of interest into pET-QINoVGII when multiple ARs containing two or more RNA fragments were required.

In several earlier studies, AR was purified using gradient ultracentrifugation process alone (Pasloske et al. 1998; Wei et al. 2008a); however, after ultracentrifugation, the target fraction was rarely as obvious as was anticipated. Fractions obtained using CsCl density gradient ultracentrifugation probably contained fragments of nucleic acids or proteins of the host cell or even fragments of the residual recombinant plasmids, leading to obvious fluorescence signals in the real-time PCR (data not shown). These foreign entities seriously impact the purity, homogeneity, and creditability of AR as a reference material or positive control. In this study, AR was purified using CsCl density gradient ultracentrifugation combined with column chromatography, and the foreign entities were thus efficiently removed. In addition, the purity could be objectively and quantitatively analyzed using a spectrophotometer (Yu et al. 2008; Zhan et al. 2009) and further confirmed using real-time PCR. Thus, compared with gradient ultracentrifugation alone, our method

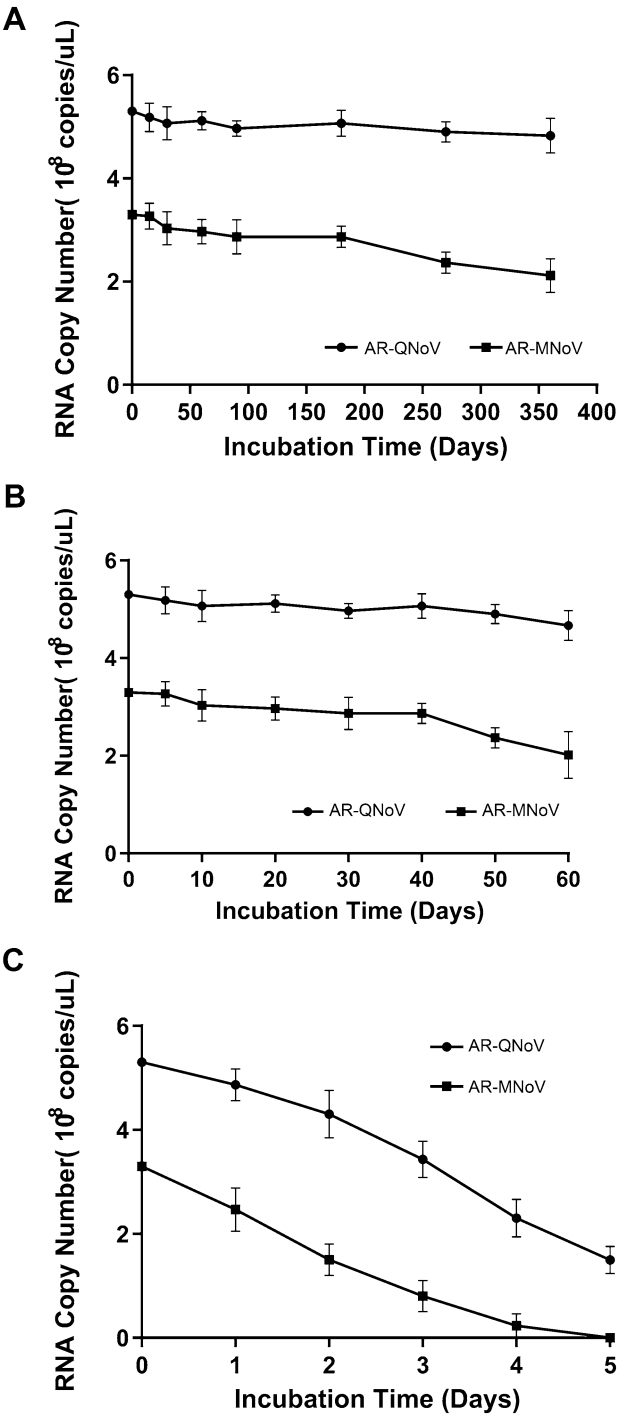

Fig. 8 Analysis of AR-QNoV and AR-MNoV stability at 4 °C for 60 days (**a**), − 20 °C for 360 days (**b**), and 45 °C for 5 days (**c**)

was easy to operate and standardize even for researchers without much experience with this technique. Moreover, the purity of AR could be controlled inexpensively, effectively, and steadily even for large-scale production, which is a crucial factor for its wider practical applications.

The stability of the Qβ VLPs is greater than that of MS2. Moreover, MS2 decomposes at ~ 15 °C, which lower than that at which Qβ decomposes, and Qβ VLPs show a robust structure (Fiedler et al. 2012). Unlike MS2, Qβ possesses two cysteine residues per coat protein, which are located at the 3- and 5-fold axes of symmetry in the quaternary structure (Stonehouse et al. 1996; Bundy and Swartz 2011). These cysteine residues link the individual monomers to either hexameric or pentameric subunits, increasing the overall thermal stability of the capsid (Ashcroft et al. 2005; Bundy and Swartz 2011; Chen et al. 2016). These characteristic of excellent thermal stability would theoretically make Qβ VLP a better strategy than MS2 for AR preparation. Commonly used temperatures during the preservation and transportation of AR were considered in this study, such as − 20 °C, which is the temperature for long-term AR storage in most laboratories, and 4 °C, which is the temperature for temporary AR storage in laboratories or during cold-chain transportation. In addition, to confirm its thermal stability, 45 °C was set as the standard temperature used to examine shipping compatibility (Pasloske et al. 1998). These results showed that under different temperatures, the rate of decline in the copy numbers of AR prepared using the Qβ packaging system developed in this study was lower than that of AR prepared using the MS2 packaging system. Such delay in the decline in copy numbers further confirmed the greater stability of AR prepared using the Qβ system.

The stability of AR prepared using the MS2 packaging system at − 20 °C or 4 °C in this study was slightly different from that published previously, and these ARs added to human plasma with sodium azide (Pasloske et al. 1998), bovine serum (Wei et al. 2008b), or Dulbecco's Modified Eagle Medium (DMEM; Sun et al. 2013) to simulate real clinical conditions. Plasma, bovine serum, and DMEM are rich in proteins, amino acid, lipids, and carbohydrates, which could sufficiently protect the MS2 protein coat and increase AR stability. Our research focused the development of a new AR packaging system using bacteriophage Qβ and compared its stability with the conventional MS2 AR. Both types of AR were diluted in RNase-free ddH_2O, but the packaging sequence in our study belonged to a norovirus rather than human immunodeficiency virus, Zika virus, and avian influenza A virus, noroviruses usually contained in stool, shellfish, vegetables, berries, and water. Thus, we did not add plasma, bovine serum, or DMEM to AR in this study.

Conclusion

In this study, we developed a novel technology to prepare AR using the bacteriophage Qβ packaging systems for the detection of norovirus (GII). The AR prepared using the developed bacteriophage Qβ packaging system was more stable than that prepared using the conventional MS2 phage system at 45 °C, − 20 °C, and 4 °C. This novel technology can provide laboratories with a positive control or reference material with increased stability, which could greatly reduce the cost and time required for periodic monitoring and evaluation.

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Compliance with Ethical Standards

Conflict of interests The authors declare that they have no competing interests.

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