Development of rapid and easy detection of Salmonella in food metrics using RPA-CRISPR/Cas12a method

Introduction (Mona Alotaibi)

1. Foodborne infections, particularly those caused by Salmonella species, pose significant public health risks and economic burdens.
2. Clinical symptoms of Salmonella infection include abdominal pain, nausea, and diarrhea, with severe cases potentially being life-threatening.
3. Regulatory bodies, including the Chinese government and the International Commission on Microbiological Specifications for Foods, set strict standards for Salmonella detection in food samples.
4. Salmonella can contaminate various food products, such as eggs, dairy, and meat, and is known for its resilience in different environments.
5. Current detection methods for Salmonella are often time-consuming, requiring 4-7 days, and are not suitable for on-site testing.
6. While nucleic acid-based methods like PCR and qPCR are effective, they involve complex procedures and expensive equipment.
7. The emergence of CRISPR technology, particularly the Cas12a enzyme, offers a promising approach for rapid and sensitive detection of Salmonella.
8. This study aims to develop a rapid, easy, and on-site detection method for Salmonella using the RPA-CRISPR/Cas12a system, addressing the limitations of existing methods.

Material and Methods (Najd Altalha)

1- Research Objective: Develop a rapid, easy detection method for Salmonella in food using **recombinant polymerase amplification** (RPA) and CRISPR/Cas12a.
2- Cas12a System Optimization: Selected a 21-base pair (bp) target sequence from the fimY gene, synthesized crRNA, and determined optimal concentrations of Cas12a, crRNA, and **fluorescent quenched** FQ-probe through experiments.
3- DNA Extraction: Used a simple boiling method for DNA extraction from chicken and egg samples.
4- Specificity and Sensitivity: Tested against various bacterial strains, evaluated sensitivity with different DNA and bacterial liquid concentrations, and found a detection limit of 10^3 CFU/mL in food samples.
5- Enrichment of Food Samples: Enhanced sensitivity by enriching samples with Salmonella bacterial liquid and incubating at 37°C for 3 hours.
6- Detection in Food Matrices: Applied the method to chicken and egg samples, with detection in one tube and fluorescence analysis using a portable transilluminator.
7- Method Verification: Verified accuracy by comparing with qPCR results according to the Chinese industry standard SN/T1059.7–2010.
8- Sample Preparation: Homogenized, portioned, and stored samples at -20°C, confirmed Salmonella-free status with qPCR.

9- Primer Design and RPA Optimization: Designed and screened six primers, selected R6 for specificity, and determined optimal primer concentration for RPA at 0.48 μM.

Results and discussion (Sarah Aldossery):

3.1. The Cas system: A 21-bp fimY gene fragment was analyzed using Cas12a, crRNA, and FQ-probe. The optimal concentration was 80 nM, with a 1:1 ratio for fluorescence. Excessive CrRNA hindered binding, and the optimal FQ-probe/Cas12a ratio was 2.5:1.

3.2. Extablish RPA–CRISPR/Cas12a method: Six primers were designed to identify Salmonella, with Primers R6 being the only pair that specifically identified the bacteria. The optimal concentration of primers was 0.48 μM, and the optimal MgOAc concentration was determined. The method was established by optimizing the Cas system and reaction mixture, showing that amplification enriches target fragments, accelerates fluorescence emission, and reduces detection time.

3.3. Method specificity and sensitivity: The RPA-CRISPR/Cas12a method effectively detects gDNA and Salmonella nucleic acid from bacteria, with visible fluorescence in accurate crRNA genotype samples. The method's detection limit is 102 CFU/mL, and it completes in 45 minutes, demonstrating good specificity and sensitivity.

3.4. Salmonella detection in food: The study investigates Salmonella detection using a simple boiling method. Chicken and egg samples were analyzed, with a detection limit of 103 CFU/mL. The method's sensitivity was enhanced by adding Salmonella bacterial liquid. The RPA-CRISPR/Cas12a method was found to be sensitive and rapid, with a higher detection limit for enriched chicken samples. The method's sensitivity can be improved by incubating Salmonella-enriched samples.

3.5. qPCR verification: The study used qPCR analysis to determine Salmonella Ct values in chicken and egg samples. The results were consistent with those obtained using the RPA-CRISPR/Cas12a method, which was more sensitive for quantifying Salmonella Ct values in enriched egg samples. The RPA-CRISPR/Cas12a method has good precision and sensitivity, similar to the qPCR method but not requiring expensive instruments, making it suitable for on-site Salmonella detection.

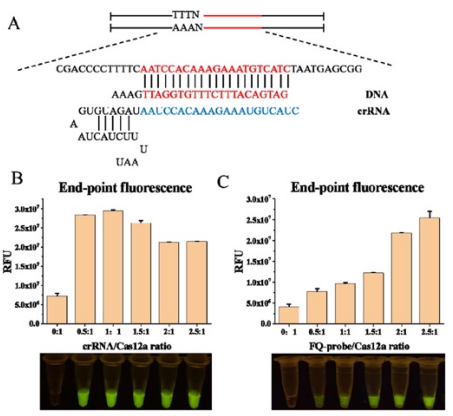

Fig. 1. Optimization of Cas system. (A) Schematic of dsDNA target detected with the Cas12a/crRNA. crRNA was provided in detail. The dsDNA target site was highlighted in red and crRNA fragments in blue. (B) Fluorescence generated from different crRNA/ Cas12a ratios. (C) Fluorescence generated from different FQ-probe/Cas12a ratios. NTC: nontarget control. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

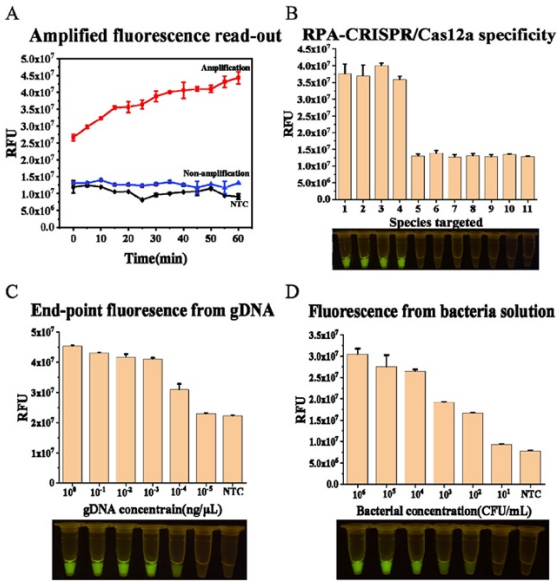

Fig. 2. Establishment and evaluation of RPA- CRISPR/Cas12a system. (A) kinetics of RPA amplifi- cation. The red square box was amplified. The blue triangular box was non-amplified. The black circular box was the blank control. (B) Specificity analysis of RPA-CRISPR/Cas12a system. Among them, the path- ogenic bacteria are as follows. 1: *Salmonella enteritidis*; 2: *Salmonella typhimurium*; 3: *Histidine - deficient Sal- monella typhimurium*; 4: *Histidine - deficient Salmonella typhimurium*; 5: *Citrobacter*; 6: *Staphylococcus aureus*; 7: *Vibrio parahaemolyticus*; *8: E. coli*; 9: *Listeria mono- cytogenes*; *10: Bacillus cereus*; 11: *nontarget control.* (C) Sensitivity analysis of RPA-CRISPR/Cas12a detection on Salmonella gDNA. (D) Sensitivity analysis of RPA- CRISPR/Cas12a detection on Salmonella microbial. NTC: nontarget control. Experiments were repeated 3 times, and at least 2 out of the 3 data sets were used for standard-deviation analysis. The standard de- viations were represented by error bars in figures. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

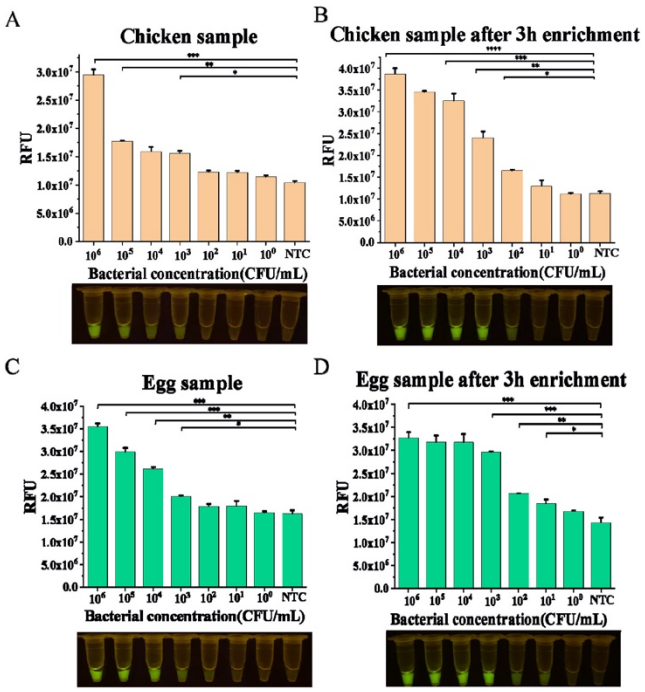

Fig. 3. Application of RPA-CRISPR/Cas12a system in real food matrix. (A) Feasibility and sensitivity anal- ysis of contaminated chicken. Provides end-point fluorescence in the food matrix. (B) Fluorescence values of chicken samples after enrichment for 3 h, and end-point

fluorescence is shown in the figure. (C) Feasibility and sensitivity analysis of contaminated eggs. Provides end-point fluorescence in the food matrix. (D) Fluorescence values of eggs sample after enrichment for 3 h, and end-point fluorescence is shown in the figure. n = 3 biological replicates, two- tailed Student's *t*-test; *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001; NTC: nontarget control. Experiments were repeated 3 times, and at least 2 out of the 3 data sets were used for standard-deviation analysis. The standard deviations were represented by error bars in figures.

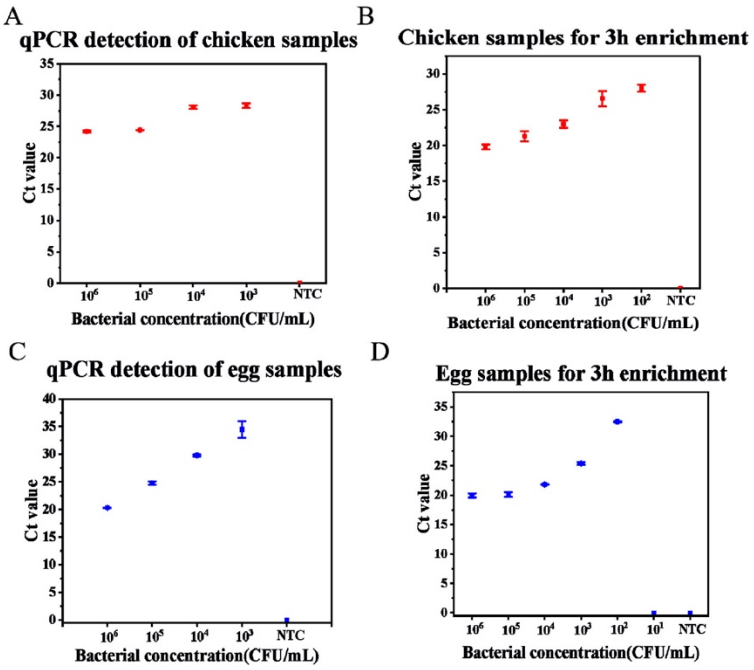

Fig. 4. Method confirmation by qPCR. (A) Ct value of salmonella from chicken sample.(B) Ct value of salmonella from chicken sample for 3 h enrichment. (C) Ct value of salmonella from eggs sample.(D) Ct value of salmonella from eggs sample for 3 h enrichment. NTC: nontarget control. Experiments were repeated 3 times, and at least 2 out of the 3 data sets were used for standard-deviation analysis. The standard deviations were represented by error bars in figures.

Conclusion (Nada alobaidi) :

The RPA-CRISPR/Cas12a method is a simple, rapid, sensitive detection method for Salmonella in food, with potential for on-site detection, cost-effectiveness, and practicality without expensive instruments or toxic dyes.

Reference:

Wen, Y., Huang, S., Lei, H., Li, X., & Shen, X. (2024). A Dual and Rapid RPA-CRISPR/Cas12a Method for Simultaneous Detection of Cattle and Soybean-Derived Adulteration in Goat Milk Powder. *Foods*, *13*(11), 1637–1637. https://doi.org/10.3390/foods13111637