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Yersinia pestis detection by loop-mediated isothermal amplification combined with magnetic bead capture of DNA

Na Feng[a,b], *Yazhou Zhou*[b], *Yanxiao Fan*[a,b], *Yujing Bi*[b], *Ruifu Yang*[b], *Yusen Zhou*[a,b,*], *Xiaoyi Wang*[a,b,*]

[a] *Anhui Medical University, 81 Meishan Road, Hefei 230032, Anhui, People's Republic of China*
[b] *Laboratory of Analytical Microbiology, State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing 100071, China*

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ABSTRACT

We developed a loop-mediated isothermal amplification (LAMP) assay for the detection of *Y. pestis* by targeting the 3a sequence on chromosome. All 11 species of the genus *Yersinia* were used to evaluate the specificity of LAMP and PCR, demonstrating that the primers had a high level of specificity. The sensitivity of LAMP or PCR was 2.3 or 23 CFU for pure culture, whereas 2.3×10^4 or 2.3×10^6 CFU for simulated spleen and lung samples. For simulated liver samples, the sensitivity of LAMP was 2.3×10^6 CFU, but PCR was negative at the level of 2.3×10^7 CFU. After simulated spleen and lung samples were treated with magnetic beads, the sensitivity of LAMP or PCR was 2.3×10^3 or 2.3×10^6 CFU, whereas 2.3×10^5 or 2.3×10^7 CFU for magnetic bead-treated liver samples. These results indicated that some components in the tissues could inhibit LAMP and PCR, and liver tissue samples had a stronger inhibition to LAMP and PCR than spleen and lung tissue samples. LAMP has a higher sensitivity than PCR, and magnetic bead capture of DNAs could remarkably increase the sensitivity of LAMP. LAMP is a simple, rapid and sensitive assay suitable for application in the field or poverty areas.

Introduction

Plague is a zoonotic disease caused by Gram-negative bacterium *Yersinia pestis*, which is occasionally transmitted to humans from *Y. pestis*-infected rodents via the bites of infected fleas.[1] *Y. pestis* is thought to have evolved from a serotype O: 1b strain of *Y. pseudotuberculosis* about 6000–10,000 years ago although these two species cause remarkably different diseases.[2–3] *Y. pestis* is a highly virulent and infectious pathogen, and was classified as a Category A pathogen by the U. S. Centers for Disease Control and Prevention.[4] Historically, *Y. pestis* has given rise to three major plague pandemics, leading to millions of human deaths. Recently, the increased outbreak of plague around the world is reported annually to

* *Corresponding authors.*
E-mails: yszhou@bmi.ac.cn (Y. Zhou), wgenome@163.com (X. Wang).
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Table 1 – Sequence of the primers for LAMP and PCR reactions.

Primer names	Type	Sequence (5′–3′)	Primer length (bp)
3a-F3	Forward outer primer	ACTACCATCCCCTCAAGGTT	20
3a-B3	Backward outer primer	GAGGGCGTTTTGGTAGAGAA	20
3a-FIP	Forward inner primer	CACCCGGCGTTATCTCATCCCG-TTTTCGAGTAGGGTTAGGTGGGC	44
3a-BIP	Backward inner primer	CATGGACGTATGGCGGGTCA-TTTTGTGATGCCGTCCAATGCA	42
3a-LF	Forward loop primer	ACCGCCATGAAATGGACAATG	21

the World Health Organization (WHO), and plague was classified as a re-emerging infectious disease by the WHO.[5–6] In addition, plague has been attracting a considerable attention because its causative agent has always been recognized as one of the classical biological warfare or bioterrorism agents.[7–8] To minimize these threats, development of a rapid method for the detection of *Y. pestis* is essential.

Y. pestis was often detected by bacterial isolation and microscopy observation,[9] phage lysis assay,[10–11] ELISA assays based on the detection of F1 antigen and antibodies against *Y. pestis*,[12–15] conventional PCR assays,[16–18] real-time quantitative PCR assays,[19–28] biosensors based on fiber-optic or upconverting phosphor technology,[29–31] solid-phase radioimmunoassay based on radiolabeled monoclonal antibody for the detection of plague antigen.[32] All these methods are playing an important role in the diagnosis of plague, but these methods either are time-consuming and laborious, or require expensive equipment and personnel with a high level of technical expertise. However, permanent surveillance of the foci of plague located in the poor regions to predict future epizootics in rodents and exposure risk for humans or investigation of samples suspected of bioterrorism on site requires a simple, rapid and efficient diagnostic method. A colloidal gold particles-based lateral-flow (LF) strip detection method for *Y. pestis* has been developed based on antibodies to F1 and LcrV proteins.[33–34] This dipstick test is a low-cost, easy-to-use and rapid screening method in the surveillance of plague or investigation of samples suspected of bioterrorism on site, but nucleic acid-based rapid detection technology could be a more powerful alternative for detecting *Y. pestis*.

Loop-mediated isothermal amplification (LAMP) technology has received considerable attention because it allows efficiently amplification of DNA with high specificity and sensitivity under isothermal conditions of 60–65 °C. The LAMP reaction can be accomplished within less than 1 h based on a set of four to six primers and the Bst DNA polymerase with strand displacement activity.[35] The method is more suitable for field applications, especially in poverty areas, because it does not require specialized or expensive equipment, and only a simple and inexpensive water bath or heating block can satisfy LAMP assay.[36–37] In addition, LAMP results can be read by the naked eye, or the lateral flow dipstick (LFD) under natural light, which makes the detection results easy to be judged in the field. Currently, the LAMP technique has been widely used in the diagnosis of infectious diseases,[38] and it is more sensitive in detecting bacteria compared to the conventional polymerase chain reaction (PCR) method.[39–40] In this study, we construct a simple and rapid LAMP method for detection of *Y. pestis* based on the specific sequence 3a (GenBank accession no. AF350075) that is a specific fragment located on *Y. pestis* chromosome found by using a comparative genomic method.[41] The specificity of the method was evaluated by using all 11 species of the genus *Yersinia*. The sensitivity was evaluated by using *Y. pestis* pure culture, and simulated tissue samples, and magnetic bead-treated simulated tissue samples.

Materials and methods

Bacterial strains, reagents, instruments and animals

Y. pestis EV vaccine strain, *Y. pseudotuberculosis*, *Y. enterocolitica*, *Y. frederiksenii*, *Y. intermedia*, *Y. kristensenii*, *Y. bercovieri*, *Y. mollaretii*, *Y. rohdei*, *Y. ruckeri*, and *Y. aldovae* were used to evaluate the specificity of LAMP and PCR; 10× thermopol buffer, $MgSO_4$ (100 mM), and Bst DNA Polymerase, Large Fragment (8000 U/ml) were purchased from NEB (Beijing, China); Calcein and real-time turbidity meter LA-320c were purchased from Eiken China CO., LTD.; Taq DNA polymerase (5 U) and dNTPs (2.5 mM) were purchased from TaKaRa (Dalian, China); SM3-P100, amino-modified silica-coated magnetic beads, was purchased form Shanghai Allrun Nano Science & Technology CO., LTD. BALB/c were obtained from Laboratory Animal Research Center, Academy of Military Medical Science, China (licensed from the Ministry of Health in General Logistics Department of Chinese People's Liberation Army, Permit No. SCXK-2007-004). The protocols were approved by Committee of the Welfare and Ethics of Laboratory Animals, Beijing Institute of Microbiology and Epidemiology. The experiments were conducted strictly in compliance with the Regulations of Good Laboratory Practice for nonclinical laboratory studies of drug issued by the National Scientific and Technologic Committee of People's Republic of China.

LAMP and PCR assays

For LAMP reaction, a set of five primers was designed according to the published sequence of 3a in *Y. pestis* KIM D46 (GenBank accession no.: AF350075) using Primer Explorer version 4. A forward inner primer (FIP), a backward inner primer (BIP), two outer primers (F3 and B3), and a loop forward primer (LF) were used for LAMP amplification. The sequences of the primers are shown in Table 1. LAMP reaction was performed in a total volume of 25 μL mixture containing 10× Thermopol buffer (2.5 μL), 100 mM $MgSO_4$ (1.5 μL), 2.5 mM dNTP Mix (14 μL), 100 mM 3a-FIP (0.4 μL), 100 mM 3a-BIP (0.4 μL), 10 mM 3a-F3 (0.5 μL), 10 mM 3a-B3 (0.5 μL), 10 mM 3a-LF (0.2 μL), 8000 U/mL of Bst DNA Polymerase, Large Fragment (1 μL), water (3 μL), template DNA (1 μL). The LAMP reaction was carried out at 65 °C for 60 min and inactivated at 80 °C for 5 min in water bath. For direct visual detection of DNA

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