Journal of Microbiological Methods 158 (2019) 18–20

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Short communication

Rapid detection of *Lactobacillus crispatus* and *Lactobacillus iners* in vaginal specimens by loop-mediated isothermal amplification

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ARTICLE INFO

Keywords:
Bacterial vaginosis
Lactobacillus crispatus
Lactobacillus iners
LAMP
Loop-mediated isothermal amplification
Rapid detection

ABSTRACT

A rapid detection method for *Lactobacillus crispatus* and *Lactobacillus iners*, which are important for maintaining a healthy vaginal environment, was developed using loop-mediated isothermal amplification (LAMP). The LAMP assay had a lower limit of detection of 10 fg DNA and could detect both species within 45 min.

The vaginal microbiome is generally dominated by *Lactobacillus* species (Amabebe and Anumba, 2018), which are important for maintaining a healthy vaginal environment. Their metabolic products, such as hydrogen peroxide, lactic acid, and bacteriocins, inhibit invasion of external pathogens (Muzny et al., 2018). *Lactobacillus crispatus*, *L. iners*, *L. jensenii*, and *L. gasseri* predominate the vaginal microbiome of healthy women. Replacement of *Lactobacillus* species by overgrowth of *Gardnerella vaginalis*, *Atopobium vaginae*, *Prevotella*, *Peptostreptococcus*, and/or *Bacteroides* leads to bacterial vaginosis (BV). Therefore, the rapid and accurate detection of health- and BV-related microorganisms is clinically important. In the clinic, vaginal microorganisms are identified morphologically by Gram staining.

During the last two decades, rapid and simple loop-mediated isothermal amplification (LAMP) assays have been used in the clinic and the food industry to detect pathogens. LAMP rapidly amplifies nucleic acids with high specificity and sensitivity under isothermal conditions (Notomi et al., 2000) using self-recurring strand-displacement synthesis and a specially designed set of target-specific primers. The results of LAMP can be read visually.

In this study, we developed an LAMP assay for *L. crispatus* and *L. iners* in vaginal specimens. One set of four specially designed primers, which recognize six distinct target sequences, were used for the assay. The sequences of the inner primers were specific for the sense and antisense strands of the target sequence. Our LAMP method uses loop primers to achieve reaction times that are less than half of those of the original LAMP method (60 min) (Notomi et al., 2000).

The LAMP primers targeted the 23S rRNA gene of *L. crispatus* (GenBank accession number; NR_077026) and *L. iners* (NZ_GG700803), and were designed using Primer Explorer v. 5 software (https://primerexplorer.jp/lampv5/index.html). The specificities of the LAMP primers were confirmed by BLAST searching. LAMP was performed in a total reaction volume of 25 µL containing 40 pmol of each inner primer (FIP and BIP), 5 pmol of each outer primer (F3 and B3), 20 pmol loop primers (LF or LB) (Table 1), 1.4 mmol of each deoxynucleotide triphosphate, 20 mM Tris–HCl (pH 8.8), 10 mM KCl, 10 mM $(NH_2)SO_4$, 8 mM $MgSO_4$, 0.1% Triton X-100, and 8 U Bst DNA polymerase large fragment (Nippon Gene, Toyama, Japan), to which 1 µL Fluorescent Detection Reagent (Eiken Chemical) and 1 µL template DNA were added. The template DNA was denatured at 95 °C for 5 min and chilled on ice. The mixture was incubated in a PCR Thermal Cycler Dice Gradient (Takara Bio, Shiga, Japan) at 65 °C for 45 min and heated at 80 °C for 5 min to terminate the reaction.

To determine the detection limit, tenfold serial dilutions of *L. crispatus* and *L. iners* DNA (1 ng to 1 fg) were prepared. LAMP products were assessed visually in natural light by a color change to green, by color development under ultraviolet (UV) light, and a ladder-like pattern by agarose gel electrophoresis. The detection limit of the LAMP system was 10 fg DNA for both species (Fig. 1).

Abbreviations: BV, bacterial vaginosis; LAMP, loop-mediated isothermal amplification; PCR, polymerase chain reaction; qPCR, quantitative PCR; UV, ultraviolet

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https://doi.org/10.1016/j.mimet.2019.01.011
Received 1 October 2018; Received in revised form 18 January 2019; Accepted 19 January 2019
Available online 22 January 2019

Table 1
Primers used in this study.

Target	Primer	Sequence (5′ to 3′)	Length (bp)
L. crispatus 23S rRNA	F3	ACGAGTTGTGAAGAGGAGTGA	21
	B3	TGTTTGGGCCTATTCACTGC	20
	FIP (F1c-F2)	CGGTTTGCGGTACGGGTATGTCGTGTGGTAATCACACTGCCA	42
	BIP (B1c-B2)	AGGAACTCGGCAAAATGACCCCGGCTAACCAATCTCTTGGCT	42
	LB	TAACTTCGGAAGAAGGGGTGCT	22
L. iners 23S rRNA	F3	GACTCGCTTTCGCTTCGG	18
	B3	CCAGTGACCGATAGTGAACC	20
	FIP (F1c-F2)	AAGTGTGATGGCGTGCCTTTTGTTGACTTAACCTTGCACCACA	43
	BIP (B1c-B2)	ACTTGTAGGCACACGGTTTCAGGAGTACCGTGAGGGAAAGGT	42
	LF	TGAACCGGCGAGTTGCG	17
	LB	TTCTCTTTCACTCCCCTCCC	20

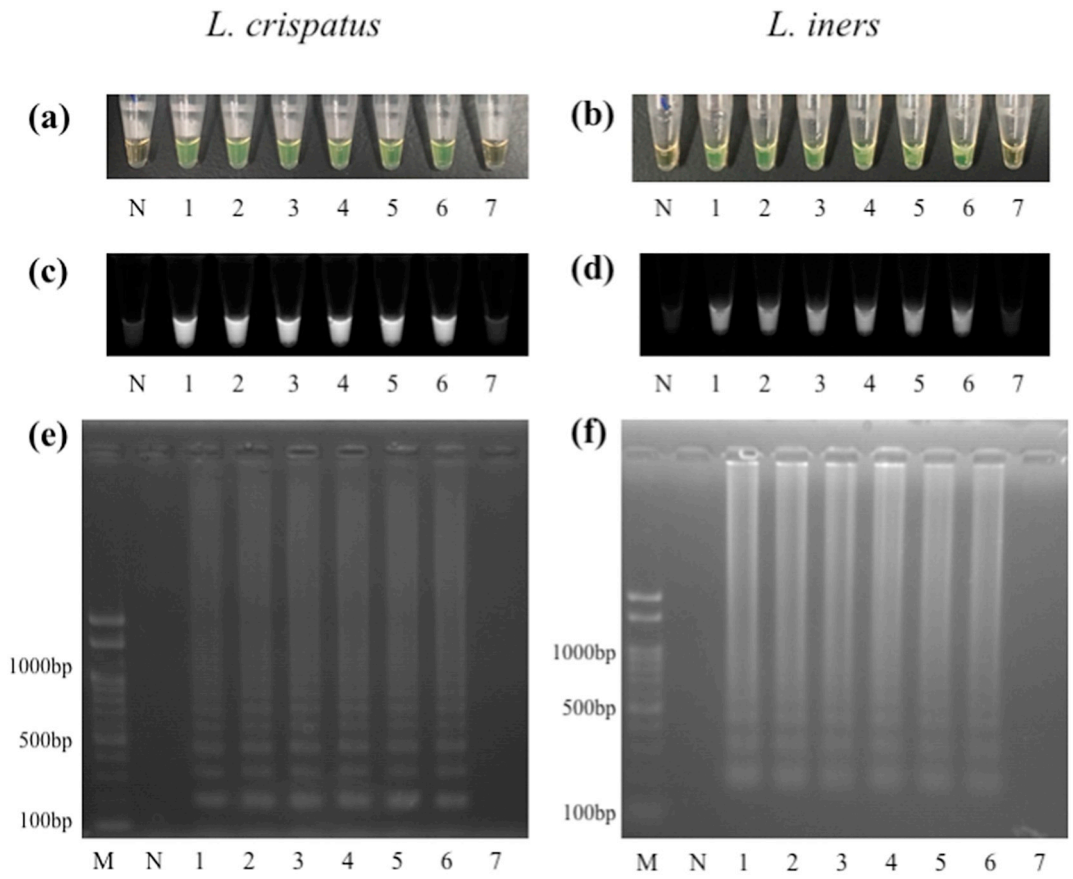

Fig. 1. Visual detection, UV detection, and agarose gel electrophoresis of LAMP products.
N, No template; 1, 1 ng; 2, 100 pg; 3, 10 pg; 4, 1 pg; 5, 100 fg; 6, 10 fg; and 7, 1 fg.
(a) (b) Color change to green.
(c) (d) Color development under ultraviolet (UV) light.
(e) (f) Ladder-like pattern following agarose gel electrophoresis of LAMP products. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

We detected *L. crispatus* and *L. iners* in vaginal samples of healthy women using the LAMP assay. Sixty healthy Japanese women with no gynecological history (mean age, 29.2 ± 5.4 years) were involved in this study, from whom vaginal samples were obtained by swabbing. The study protocol was approved by our Institutional Review Board (nos. Rin 10–14 and Rin 11–25). Microbial DNA was extracted from the swabs by a previously described method (Higashide et al., 2018). Among the 60 samples, 42 and 38 were positive for *L. crispatus* and *L. iners*, respectively. The positive reactions were confirmed by DNA sequencing, i.e., by matching the sequence of each amplicon of the type strain of the corresponding species. We previously developed a rapid LAMP detection system for *Gardnerella vaginalis* in vaginal samples; therefore, both health- and BV-related microorganisms can be detected by LAMP. The detection limit, 10 fg DNA, corresponds to 4.4 and 7.1 copies of the genomes of *L. crispatus* and *L. iners*, respectively, which is similar to the sensitivity of qPCR. LAMP thus represents an alternative to culture-based or morphological detection methods. For example, although direct microscopic observation of sputum smears is used for definitive diagnosis of tuberculosis, the WHO recommends an LAMP assay (http://www.who.int/tb/publications/lamp-diagnosis-molecular/en/). The LAMP systems developed in this and prior studies will facilitate on-site monitoring of premature birth, for which BV is a causative factor.

In conclusion, we developed an LAMP system for detecting *Lactobacillus* species, and plan to evaluate its utility using clinical samples from patients with BV.

Conflicts of interest

None.

Funding

This work was supported in part by a grant (to TS) from the Japan Society for the Promotion of Science (Grant No. 16K08384).

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