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EDITED BY
Maria L. Inácio,
National Institute for Agricultural and Veterinary Research (INIAV), Portugal

REVIEWED BY
Alba Pacheco Moreno,
John Innes Centre, United Kingdom
Svetlana Khmeleva,
Institute of Biomedical Chemistry, Russia

*CORRESPONDENCE
Firas Ali Ahmed
firasa.alrekaby@uokufa.edu.iq

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Development of a robust, deployable loop-mediated isothermal amplification assay for rapid and specific detection of soft rot caused by *Pectobacterium*

Hassan Ali Tamur[1,2] and Firas Ali Ahmed[1]*

[1]Department of Plant Protection, Faculty of Agriculture, University of Kufa, Al Najaf, Iraq, [2]Al-Qasim Green University, College of Biotechnology, Applied Biotechnology Department, Babylon, Iraq

Soft rot, caused by the phytopathogenic bacteria *Pectobacterium* and *Dickeya*, is a serious and destructive disease of potato worldwide. Symptoms caused by the two genera are indistinguishable. An effective diagnostic strategy is essential for controlling the disease and reducing losses in potato production. In this study, we examined genomes from multiple *Pectobacterium* and *Dickeya* species and selected the distinctive p4h gene to design specific primers and develop a loop-mediated isothermal amplification (LAMP) assay for the rapid and specific detection of *Pectobacterium* among other genera causing soft rot. The assay was designed for use with a field-portable thermal flask at 65 °C. The specificity of the developed LAMP assay was evaluated using 15 bacterial strains comprising inclusivity and exclusivity panels. The assay detected as little as 5 fg (~9000 genomic copies) of genomic DNA. It also detected *Pectobacterium* in infected potato tuber samples. Results were consistent between laboratory and field conditions, with no false-positive or false-negative results. The developed LAMP assay may be applied to diagnostics, agricultural quarantine, and disease management.

KEYWORDS
blackleg, diagnosis, LAMP, portable technique, potato

Introduction

Soft rot of potato (*Solanum tuberosum* L.) is one of the most serious and economically important diseases affecting a range of crops, including potato, tomato, maize, pineapple, carrot, sugar beet, and several ornamental plants (Ma et al., 2007; Samson et al., 2005). The disease is caused by the closely related genera *Pectobacterium* and *Dickeya* (formerly *Erwinia* in the family *Enterobacteriaceae*) and results in pre- and postharvest losses (Werra et al., 2015; Li et al., 2020). The genus *Pectobacterium* encompasses various species that cause wilting, stem rot, blackleg, maceration, and postharvest diseases in numerous fruit and vegetable crops. Particularly harmful species include *P. carotovorum*, *P. atrosepticum*, *P. betavasculorum*, *P. wasabiae*, *P. brasiliense, P. punjabense, and P. polaris*, which affect both monocot and dicot hosts (Larrea-Sarmiento et al., 2018). Soft rot causes greater quantitative

losses than any other bacterial disease. Global crop losses caused by soft rot exceed $420 million annually, with postharvest losses estimated at approximately 20%–30% of harvested produce (Babinska-Wensierska et al., 2024; Bhat et al., 2010). *Pectobacterium* and *Dickeya* share a common host range, and the symptoms they cause are indistinguishable (Almasi, 2019). Some studies have reported that soft rot pathogens survive as mixed populations in infected tuber tissue (van der Wolf et al., 2021). Differentiating *Pectobacterium* from *Dickeya* in infected plant tissues is therefore challenging because pathogens in these genera cause similar symptoms on the same hosts. A rapid diagnostic tool is needed to distinguish *Pectobacterium* from *Dickeya* and related genera in the family Enterobacteriaceae to monitor the epidemiology of these infections and identify sources of contamination.

Conventional bacteriological methods are time-consuming and require specialized skills for routine genus-level identification. Although the polymerase chain reaction (PCR) is currently the most widely used technique for identifying plant pathogens (Li et al., 2011), it is time-consuming, costly, and not readily deployable in the field. Several isothermal amplification methods are currently available, including strand displacement amplification (SDA) (Little et al., 1999), recombinase polymerase amplification (RPA) (Ahmed et al., 2018), helicase-dependent amplification (HDA) (Vincent et al., 2004), nicking enzyme amplification reaction (NEAR) (Van Ness et al., 2013), loop-mediated isothermal amplification (LAMP) (Palanisamy et al., 2025), and rolling circle amplification (RCA) (Dean et al., 2001; Zhang and Tanner, 2017). LAMP is among the most widely used isothermal detection techniques because of its speed, simplicity, high sensitivity, and compatibility with various detection chemistries. Importantly, it can be performed at the point of care. It has been successfully used for rapid and specific diagnosis of plant-pathogenic bacteria in infected plant tissues and soil (Larrea-Sarmiento et al., 2018). Studies have also demonstrated direct LAMP detection of soft rot caused by *P. parmentieri* in infected potato and soil samples (Domingo et al., 2021).

LAMP uses a strand-displacement mechanism facilitated by a DNA polymerase, together with a pair of inner bipartite primers (FIP and BIP) and two outer primers (F3 and B3) (Nagamine et al.,

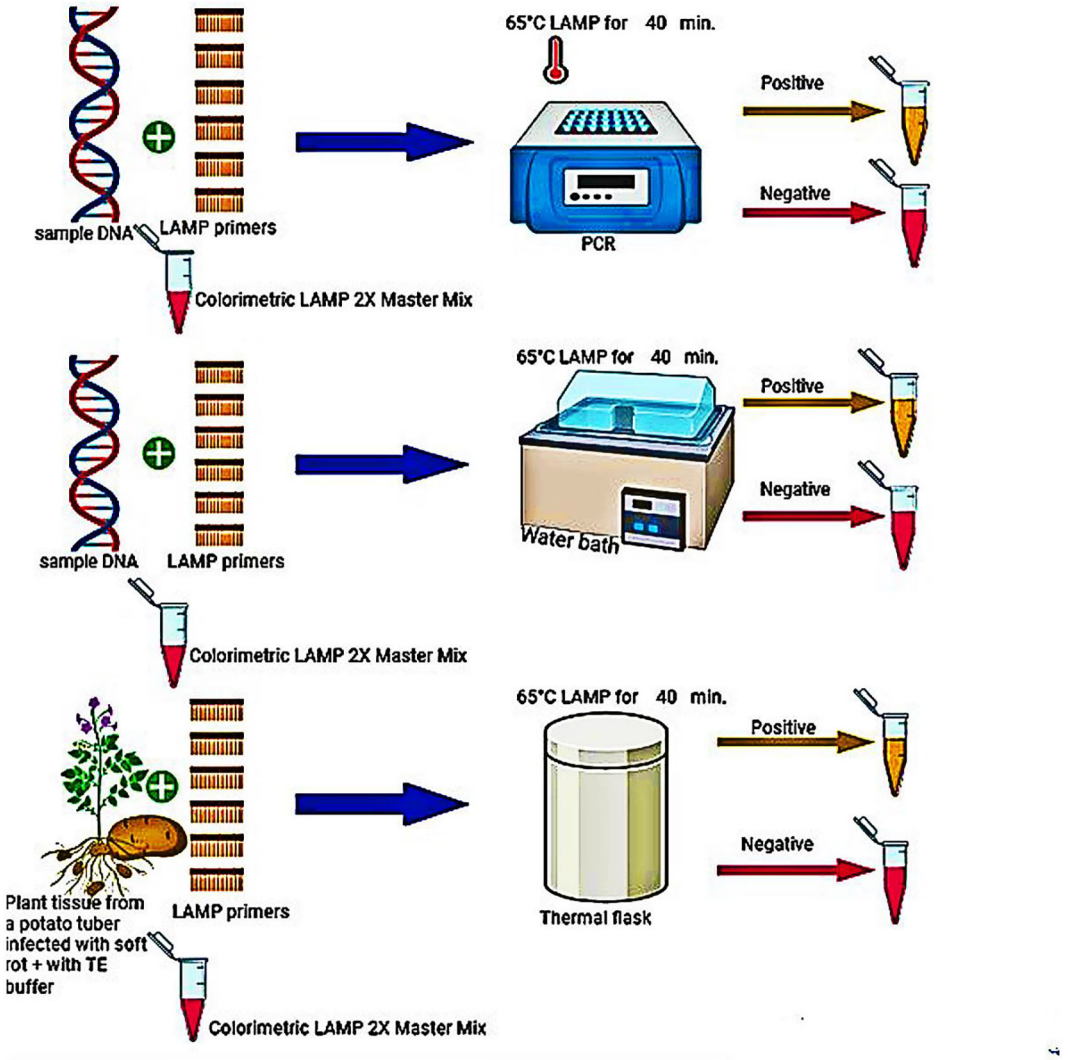

FIGURE 1
Workflow of the developed LAMP assay incorporating phenol red as a pH-sensitive indicator for colorimetric visual detection. Reaction mixtures were incubated at 65 °C for 40 min using a PCR thermal cycler, water bath, or thermal flask.

2002). The reaction begins when an inner primer (FIP or BIP) hybridizes to its corresponding priming site (F2c or B2c) on the target DNA. Subsequently, the outer primer (F3 or B3) hybridizes to its respective primer-binding site (F3c or B3c) on the target DNA, initiating synthesis of a new complementary DNA strand that displaces the extension product from the inner primer. The resulting product can form stem-loop structures at both termini. Incorporating internal loop primers (LF and LB) increases the efficiency of the LAMP reaction and reduces the overall reaction time (Kubota et al., 2008). A pH-dependent color change in phenol red enables visualization of the amplified double-stranded DNA products.

In the present study, we developed a rapid, field-deployable colorimetric LAMP assay incorporating phenol red as a pH-sensitive indicator for detecting *Pectobacterium* in purified DNA and infected plant tissues (Figure 1). The established protocol may be useful in phytopathology for routine diagnostics, monitoring, biosecurity, epidemiological studies, and disease management.

Materials and methods

Collection of diseased tuber samples and bacterial isolation

Samples of infected potato tubers were collected from six Iraqi provinces: Babylon, Baghdad, Karbala, Dohuk, Diwaniyah, and Najaf. In total, 90 samples were randomly collected from the fields (15 samples from three fields per province). Disease incidence ranged from 10% to 50%. Samples were surface-sterilized with 1% sodium hypochlorite for 3 min, rinsed three times with sterile water, and cut into small pieces that were plated on nutrient agar medium (NutriSelect Plus, Sigma-Aldrich, St. Louis, MO, USA) (Dana et al., 2015). Plates were incubated for 24 h at 28 °C, and single bacterial colonies were transferred to crystal violet pectate (CVP) medium and incubated under the same conditions (Abdelghany et al., 2022). Cavity formation on CVP medium indicated *Pectobacterium* (Kado, 2010). Pathogenicity was confirmed on whole potato tubers of the Arizona cultivar. A sterile toothpick dipped in a bacterial suspension with an OD600 (Optical Density at 600 nm) of approximately 0.1 was used to stab-inoculate healthy potato tubers. Inoculated tubers were incubated for 48 h to allow symptoms to develop. The following bacteriological tests were performed on each strain: oxidation/fermentation (O/F), catalase, growth on nutrient agar at 37 °C, KOH, and indole production. For the oxidation/fermentation test, two tubes containing sterile O/F green medium (Thermo Fisher Scientific, Waltham, MA, USA) were used for each bacterial strain. One tube (oxidative) was exposed to air, and the other (fermentative) was overlaid with sterile mineral oil to create anaerobic conditions. Both tubes were inoculated with the bacterial strain being tested. For oxidative strains, only the open tube changes from green to yellow, whereas the closed tube remains green (Arbefeville et al., 2024). By contrast, both tubes turn yellow for fermentative strains. For the catalase test, one drop of 3% hydrogen peroxide (H_2O_2) was placed on a clean glass slide. A colony from a 24-h bacterial culture was picked with a sterile toothpick and applied to the hydrogen peroxide. Immediate vigorous bubbling indicated a positive result. For the indole production test, tubes containing sterile tryptophan broth medium (Sigma-Aldrich, St. Louis, MO, USA) were inoculated with a loopful of a 24-h pure bacterial culture. The tubes were incubated at 35 °C for 24 h, after which 0.5 mL of Kovac's reagent was added directly to each tube. The tubes were gently shaken to allow the reagent to rise to the top. Formation of a red or pink ring at the top of the tube within seconds indicated a positive reaction, whereas a yellow upper layer indicated no indole production (MacFaddin, 2000). The KOH string test was conducted by mixing a bacterial colony with one drop of 3% KOH on a clean slide for 30 s. The formation of a viscous thread indicated a Gram-negative reaction (Schaad et al., 2001).

Genomic DNA extraction and identity confirmation

Bacterial genomic DNA was extracted from pure bacterial cultures using a microbial DNA purification kit (Promega, Madison, WI) according to the manufacturer's instructions. DNA was quantified using a NanoDrop spectrophotometer. DNA from all bacterial strains was amplified by polymerase chain reaction (PCR) using primers targeting the *dnaA* (Schneider et al., 2011) and 16S rRNA (Dobhal et al., 2016) gene regions. PCR conditions for the *Pectobacterium* sp. *dnaA* primers were as follows: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 61 °C for 1 min, and extension at 72 °C for 30 s; and final extension at 72 °C for 4 min. PCR products were electrophoresed on a 1.5% agarose gel, stained with 0.4 µg/mL Safe DNA Gel Stain (APExBIO, Houston, USA), and visualized under UV illumination. For purification, 4 µL of ExoSAP (Macrogen, Seoul, South Korea) was added to 10 µL of PCR product. Samples were incubated at 38 °C for 12 min and then at 80 °C for 12 min to inactivate the enzyme. Sanger sequencing was performed at the Macrogen facility. Raw forward and reverse reads were aligned using Geneious Prime 2026.0, and the alignments were manually assessed for errors. Consensus sequences were generated, and identities were confirmed by searching the NCBI GenBank nucleotide and genome databases using BLASTn. Pairwise sequence similarity among strains was calculated using Sequence Demarcation Tool v1.2.

Optimization of LAMP assay conditions for detection of soft rot-causing pathogens

Six LAMP primers, including a forward loop primer (FLP) and reverse loop primer (LRP), were designed using Primer Explorer V5 (https://primerexplorer.jp/e/) for the prolyl 4-hydroxylase (*p4h*) subunit of *Pectobacterium* sp. (NCBI id: NR_116047.1) (Table 1). Amplification temperatures of 60–65 °C and different incubation times were evaluated to optimize the LAMP reaction. After the reaction mixture was incubated at 65 °C for 40 min, results were visually assessed using phenol red as a pH indicator that changes color according to solution acidity. A change from pink or red to yellow at pH <6.8 indicated amplification of the target pathogen.

TABLE 1 LAMP primer sequences.

Primer name	Sequence (5′–3′)	Length (nt)	GC%	BLAST results			Source
				Query	E-value	Identity	
P4H-F3	CATGAAACCCCGTTCCAGT	19	57.80	100	0.26	100	This study
P4H-B3	AAGGCGTCAGAGGTCAGC	18	61.00	100	0.26	100	This study
P4H-FIP	CGCCGTTACGTTCACGGTAGTTTATGGCG TAACAGCAGCATC	42	52.30	100	0.016	100	This study
P4H-BIP	TTCCTTTAGCCTCCGGCAAAGTTCGTTACACA TTCCCAGCC	41	51.20	100	0.026	100	This study
BLP-Loop	GGAACTCATGGGCAAGCG	18	61.00	100	0.26	100	This study
RLP- Loop	GCGTCCTCTGGCTTAAAT	18	61.00	100	0.26	100	This study

LAMP reaction

LAMP reactions were performed in a final volume of 25 µL comprising 2 µL of primer mixture (0.2 µM each P4H-F3/B3, 0.4 µM each P4H-LF/LB, and 1.6 µM each P4H-FIP/BIP per reaction), 15 µL of WarmStart Colorimetric® Master Mix (Biolabs, Massachusetts, USA), 2 µL of template DNA, and 6 µL of molecular biology-grade water. Reactions were incubated at 65 °C for 40 min using a Techne PCR thermal cycler (Techne, ALT, USA). Results were assessed visually by color change: yellow indicated a positive reaction, and red indicated a negative reaction.

Specificity validation and sensitivity of the LAMP assay

Specificity of the developed LAMP *assay* was *evaluated* using 11 strains of *P. brasiliense*, *P. zantedeschiae*, *P. wasabiae*, *P. carotovorum*, *P. atrosepticum*, *P. odoriferum*, *P. punjabense*, *P. aroidearum*, *P. quasiaquaticum*, *P. peruviense*, *and P. polaris* as *the* inclusivity panel. *The exclusivity panel comprised Pseudomonas syringae*, *Xanthomonas campestris*, *Dickeya zeae*, and *Dickeya diantthicola* (Table 2). For sensitivity *testing*, the LAMP assay was evaluated using serial dilutions of purified *Pectobacterium genomic DNA* from 100 fg to 5 fg.

LAMP assay using a water bath without a PCR thermal cycler

LAMP reactions were prepared as described above. The reaction mixture was incubated at 65 °C for 40 min in a digital water bath (Thermo Fisher Scientific ™, MA, USA). Results were assessed visually by color change: yellow indicated a positive reaction, and red indicated a negative reaction. A no-template control (NTC; water) was included in each run to verify assay validity.

LAMP testing of infected potato tubers without DNA extraction using a thermal flask

Healthy potato tubers were sterilized by submersion in 3% NaClO for 2 min, followed by two rinses with sterile water. The tubers were artificially inoculated individually with *P. carotovorum* or *Dickeya zeae* using 15 µL of a bacterial suspension at a concentration of 10^8 Colony Forming Unit (CFU)/mL. A sterile knife was used to cut the tubers into thin slices. The potato slices were placed in Petri dishes containing filter paper moistened with 1–2 mL of sterile water to maintain humid conditions. Using a sterile toothpick, *P. carotovorum* or Dickeya zea colonies were inserted into the center of each potato slice. The inoculated slices were incubated at 28 °C for 24 h. For sample preparation, 20–50 mg of rotted tissue was mixed with 500 µL of TE buffer and macerated in an Eppendorf tube with a pestle for 2 min. The tubes were then left at 24 °C for 10 min to allow debris to settle. A 25-µL LAMP reaction comprised 2 µL of primer mix (0.2 µM each P4H-F3/B3, 0.4 µM each P4H-LF/LB, and 1.6 µM each P4H-FIP/BIP per reaction), 15 µL of WarmStart Colorimetric® Master Mix, 2 µL of supernatant from the macerated tissue as template DNA, and 6 µL of molecular biology-grade water. The reaction mixture was incubated at 65 °C for 40 min in a thermal flask (Figure 1). Results were assessed visually by color change: yellow indicated a positive reaction, and red indicated a negative reaction.

Results

Pathogenicity test and biochemical characterization of isolated bacterial strains

Eleven of the 90 bacterial strains were identified as *Pectobacterium* species and caused tissue maceration and typical soft rot symptoms in inoculated potato tubers during pathogenicity testing. These 11 strains, *Pectobacterium brasiliense, P. wasabiae, P. atrosepticum, P. carotovorum, P. odoriferum, P. aroidearum, P. quasiaquaticum, P. peruviense, P. punjabense, P. polaris*, and *P. zantedeschiae*, were selected for the inclusivity panel used to develop the LAMP assay. Previous studies have shown that bacteria can survive and grow saprophytically on potatoes and in the rhizosphere (Agrios, 2005; Czajkowski et al., 2015). In this study, representative strains of *Pseudomonas syringae*, *Xanthomonas campestris*, *Dickeya zeae*, *D. dianthicola*, and the 11 *Pectobacterium* strains (Table 2) were differentiated and confirmed by biochemical testing. Specifically, strains of *Pectobacterium* and *Dickeya* were differentiated from *Xanthomonas* and *Pseudomonas* based on cavity formation on crystal violet pectate (CVP) medium,

TABLE 2 Bacterial isolates used in the study, their GenBank accession numbers, and LAMP results.

Bacterial species	Host	Origin	Disease severity	Biochemical test results						GenBank accession number	LAMP results
				CVP	O/F	Gram[a]	Catalase	Indole production	Growth on NA at 37 °C		
Pectobacterium brasiliense	Potato	Babylon	78%	+	+/+	_	+	_	+	_	+
P. wasabiae	Potato	Babylon	80%	+	+/+	_	+	_	_	PV739280.1	+
P.atrosepticum	Potato	Babylon	47%	+	+/+	_	+	_	_	PV746269.1	+
P.carotovorum	Potato	Babylon	89%	+	+/+	_	+	_	+	_	+
P.odoriferum	Potato	Karbala	47%	+	+/+	_	+	_	+	PV746265.1	+
P.aroidearum	Potato	Karbala	40%	+	+/+	_	+	_	+	PV746253.1	+
P.quasiaquaticum	Potato	Karbala	43%	+	+/+	_	+	_	+	PV731277.1	+
P.peruviense	Potato	Al Muthanna	60%	+	+/+	_	+	_	+	PV731271.1	+
P. punjabense	Potato	Karbala	66%	+	+/+	_	+	_	+	PV731278.1	+
P. polaris	Potato	Najaf	80%	+	+/+	_	+	_	+	PV731279.1	+
P.zantedeschiae	Potato	Najaf	48%	+	+/+	_	+	_	+	PV731280.1	+
Pseudomonas syringae	Tomato	Najaf		+	+/+	_	+	+	+	_	+
Xanthomonas campestris	Cauliflower	Najaf	_	+	_	_	+	+	_	_	_
Dickeya diantthicola	Potato	Najaf	_	+	_	_	+	+	_	_	_
D. zeae	Potato	Najaf	_	+	+	_	+	+	_	_	_
Distilled water only											_

(+) indicates a positive reaction; (−) indicates a negative reaction; a) indicates the Gram reaction determined by formation of a sticky strand 30 s after adding KOH, indicating Gram-negative bacterial cells.

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oxidative/fermentative and catalase reactions, and growth on nutrient agar at 37 °C. *P. wasabiae* and *P. atrosepticum* were exceptions and did not grow at 37 °C (Li et al., 2011). Additionally, *Pectobacterium* species were distinguished from the other genera by their inability to produce indole acetic acid.

Molecular confirmation of bacterial identity

Molecular identification of the 11 bacterial strains used in the inclusivity panel to develop the *Pectobacterium* LAMP assay is shown in Table 2. *Pectobacterium* species identities were confirmed by sequencing both strands of double-stranded DNA. Sequence similarities were verified using the NCBI BLASTn database. The accession numbers obtained for sequences submitted during this study were PV739280.1, PV746269.1, PV746265.1, PV746253.1, PV731277.1, PV731271.1, PV731278.1, PV731279.1, and PV731280.1. Other strains in the inclusivity and exclusivity panels were obtained and molecularly confirmed in previous studies conducted in the laboratory (Table 2). Pairwise sequence identity among the *Pectobacterium* species used in this study ranged from 57% to 100%. The *D. Zeae* sequence showed >95% similarity to the *Pectobacterium* strains. *X. campestris* and *P. syringae* showed the greatest differences from the other strains analyzed in this study (Figure 2).

Specificity validation of the LAMP assay

The diagnostic specificity of the LAMP assay for *Pectobacterium* was evaluated using 11 *Pectobacterium* strains collected from different geographic regions in Iraq; the exclusivity panel comprised strains from other genera and species. All LAMP reactions yielded the expected results, with no false-positive or false-negative results observed. Positive results (yellow tubes) for *Pectobacterium* strains were obtained within 40 min. The primer set specifically detected only *Pectobacterium* strains (Figure 3), demonstrating that the established LAMP assay is reliable and specific for *Pectobacterium* detection.

PCR confirmation and LAMP sensitivity

The outer primers P4H-F3 and P4H-B3 were also used for conventional PCR and showed high specificity for *Pectobacterium*. Agarose gel electrophoresis showed amplification of an approximately 450-bp product in the target strains, whereas no amplification was observed in exclusivity strains or the negative control (Figure 4A). Three dilutions of purified P. carotovorum genomic DNA were assessed with the developed LAMP assay. The detection limit, indicated by a positive yellow LAMP reaction, was 5 fg. Copy number was calculated as follows: number of copies = mass in nanograms × 6.022 × 10^23/[fragment length (bp) × 10^9 × 660 g/

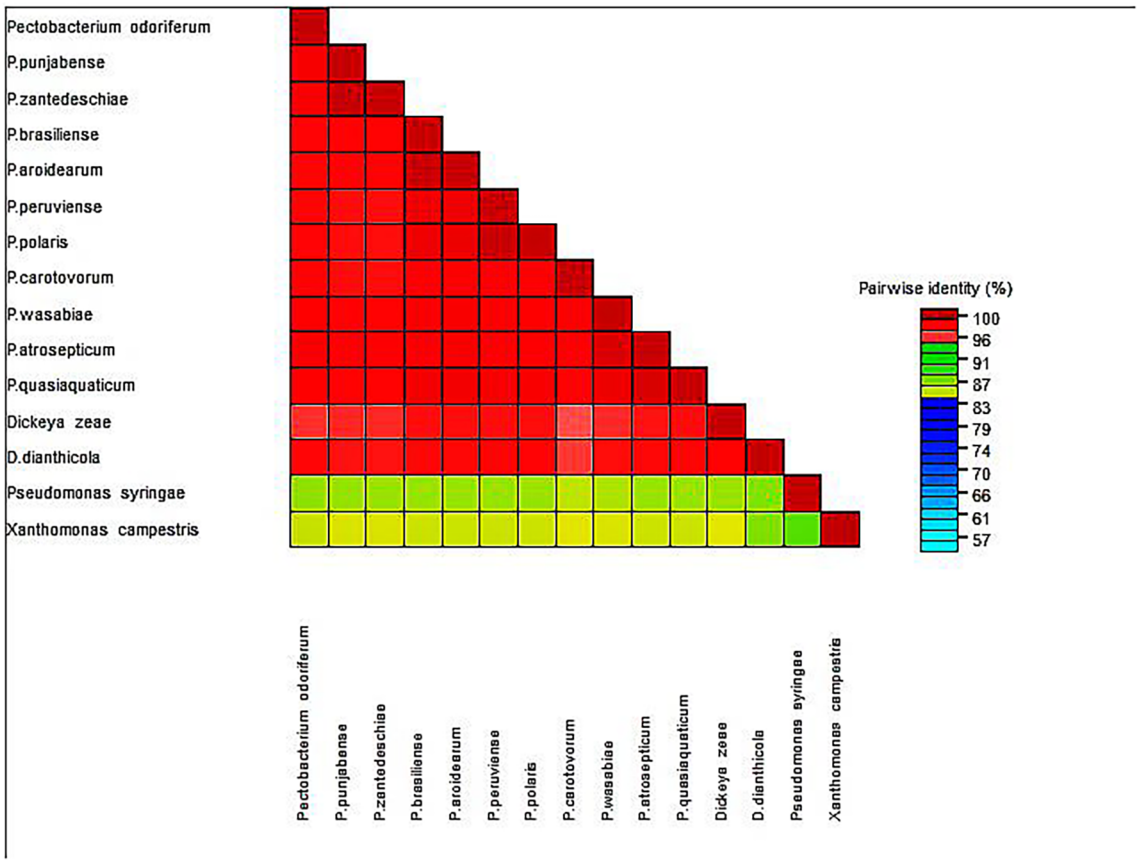

FIGURE 2
Consensus sequences of the *dnaA* gene were used to generate a color-coded pairwise identity matrix. Sequence Demarcation Tool v1.2 was used to determine percentage pairwise identity among bacterial strains in the inclusivity and exclusivity panels.

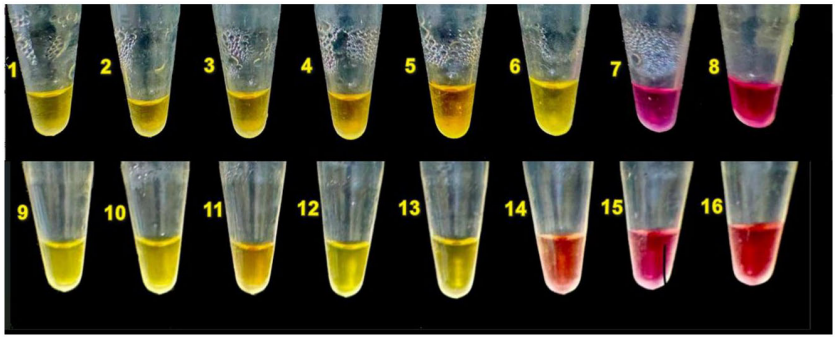

FIGURE 3
Assessment of the diagnostic specificity of the loop-mediated isothermal amplification (LAMP) assay for the target pathogen. Samples tested were: 1, *P. brasiliense*; 2, *P. zantedesthiae*; 3, *P. wasabiae*; 4, *P. carotovorum*; 5, *P. atrosepticum*; 6, *P. odoriferum*; 7, no-template control; 8, *Dickeya zea*; 9, *P. punjabense*; 10, *P. aroidearum*; 11, *P. quasiaquaticum*; 12, *P. peruviense*; 13, *P. polaris*; 14, *Dickeya diantthicola*; 15, *P. syringae*; and 16, *X. campestris*.

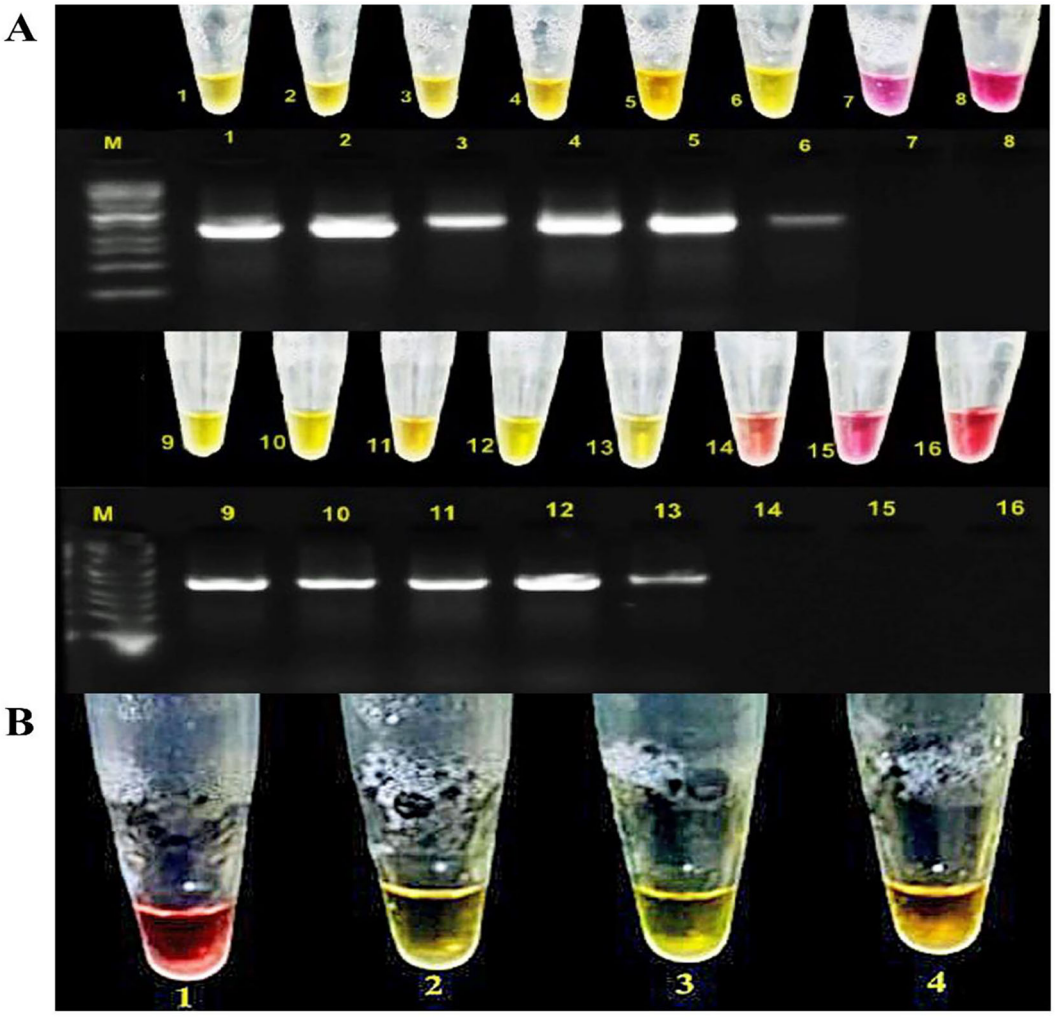

FIGURE 4
(A) Confirmation of LAMP assay specificity by agarose gel electrophoresis of conventional PCR products. M, DNA marker; 1, *P. brasiliense*; 2, *P. zantedesthiae*; 3, *P. wasabiae*; 4, *P. carotovorum*; 5, *P. atrosepticum*; 6, *P. odoriferum*; 7, no-template control; 8, *Dickeya zea*; 9, *P. punjabense*; 10, *P. aroidearum*; 11, *P. quasiaquaticum*; 12, *P. peruviense*; 13, *P. polaris*; 14, *D. diantthicola*; 15, *P. syringae*; and 16, *X. campestris*. **(B)** Sensitivity of the loop-mediated isothermal amplification (LAMP) assay using serially diluted *Pectobacterium carotovorum* genomic DNA. 1, no-template control; 2, 100 fg; 3, 50 fg; 4, 5 fg.

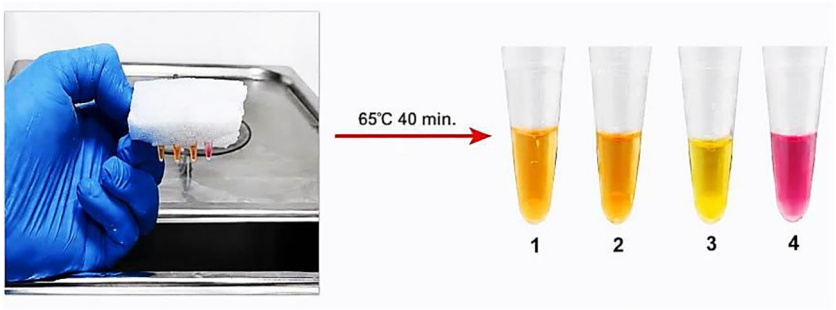

FIGURE 5
Visual assessment of LAMP products amplified in a water bath at 65 °C: 1, *P. brasiliense*; 2, *P. zantedesthiae*; 3, *P. wasabiae*; 4, *D. zeae*. Yellow indicates positive amplification of *Pectobacterium*, whereas red indicates no amplification of *D. zeae*.

mol]. Using a Pectobacterium target length of 450–500 bp and a mass of 5 fg ($5 \times 10^{\wedge -6}$ ng) yielded approximately 9000 copies. No color change was observed in the no-template control (Figure 4B).

Evaluation of the LAMP assay using a water bath

The developed LAMP assay consistently amplified *Pectobacterium* DNA without a costly PCR thermal cycler by using a water bath at 65 °C for 40 min. Positive yellow reactions were obtained with DNA from *P. carotovorum*, *P. brasiliense*, and *P. wasabiae*, whereas *D. zeae* produced a negative red reaction (Figure 5). These results demonstrate that the LAMP assay can be performed in a water bath without a PCR thermal cycler.

Expedited sample processing with TE buffer for LAMP detection using a thermal flask

Expedited sample preparation using TE buffer and a thermal flask containing distilled water at 65 °C was evaluated with infected potato tubers. Six potato tubers were artificially inoculated individually with *P. carotovorum* or *D. zeae* and then prepared for LAMP detection using TE buffer. The LAMP reaction mixture was incubated in a thermal flask at 65 °C for 40 min. Positive results were obtained from potato tubers infected with *P. carotovorum*, whereas tissue infected with D. zeae produced negative results (Figure 6). These results indicate that the LAMP assay can be used directly and rapidly for specific detection without DNA extraction or laboratory equipment.

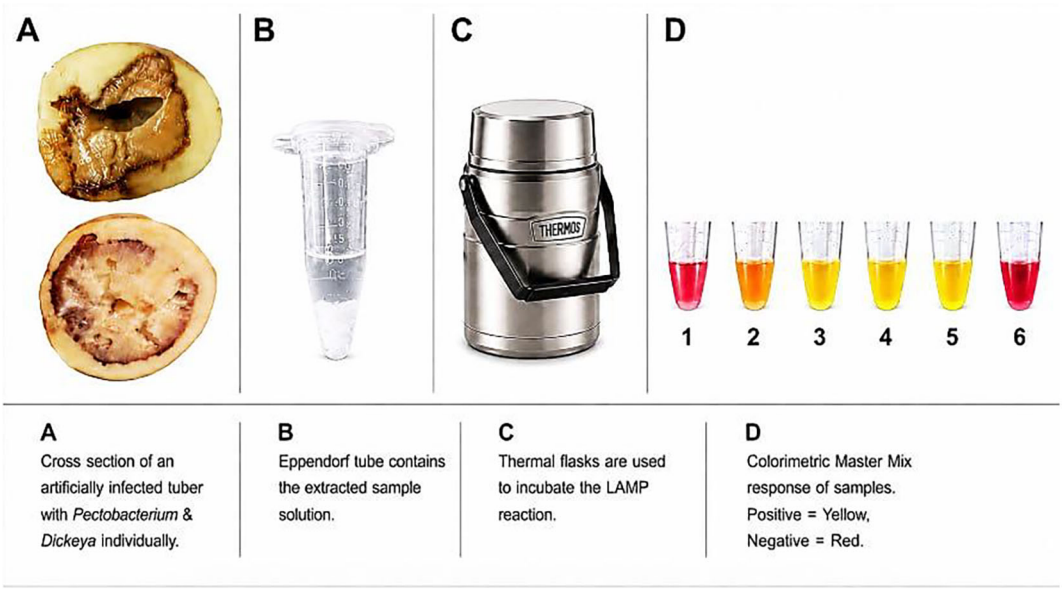

FIGURE 6
(A–D) Workflow for direct detection of *Pectobacterium* sp. in infected potato tubers using a thermal flask and rapid sample preparation with TE buffer for the LAMP assay. The results show that plant-derived inhibitors did not interfere with LAMP amplification. The simplified sample preparation procedure using TE buffer required approximately 1 min. Inoculated potato samples were evaluated using both *Pectobacterium* sp. and *D. zeae* to assess assay performance. 1, no-template control; 2, *P. brasiliense*; 3, *P. zantedesthiae*; 4, *P. wasabiae*; 5, *P. carotovorum*; 6, *D. zeae*.

Discussion

Early, reliable pathogen detection and timely decision-making are crucial for disease management. Several detection methods, including culture-based methods, ELISA, PCR, and LAMP, are available to differentiate *Pectobacterium* at the species and subspecies levels. Compared with some serological and molecular methods, LAMP can be rapidly applied in the field without a PCR thermal cycler (Huang et al., 2017). In this study, we developed a field-deployable LAMP assay that specifically detects *Pectobacterium* using a thermal flask and a 40-min reaction.

The specificity of DNA segments selected for the target plant pathogen is crucial to the effectiveness of a LAMP assay. In this study, the *p4h* (prolyl 4-hydroxylase) gene of *Pectobacterium* sp. was selected for designing pathogen-specific LAMP primers targeting the *p4h* coding region of *P. carotovorum*. By comparison, *recG* was selected as a unique gene for developing a LAMP assay for the specific and rapid detection of X. euvesicatoria, the causal agent of tomato bacterial spot (Larrea-Sarmiento et al.,2018). The alcohol dehydrogenase gene has also been selected for LAMP-based specific detection of potato blackleg caused by *D. dianthicola* (Ocenar et al., 2019).

LAMP is an efficient technique for detecting specific pathogens. Here, we demonstrated the specificity of the LAMP method for detecting soft rot pathogens by targeting 11 diverse *Pectobacterium* species. Although *Dickeya* causes similar disease symptoms and affects the same hosts, the established LAMP assay specifically detected *Pectobacterium*. The *Pectobacterium*-specific LAMP assay detected all *Pectobacterium* species tested and discriminated them from the closely related genus *Dickeya* and from other bacterial genera, including *Pseudomonas* and *Xanthomonas*. LAMP is more rapid than conventional nucleic acid-based techniques and does not require sophisticated laboratory equipment such as PCR and quantitive PCR (qPCR) instruments. LAMP results can be obtained using four core primers plus forward and backward loop primers within 30–40 min (Yasuhara-Bell et al., 2016).

The detection limit of a diagnostic technique should be sufficiently low to detect small quantities of the target pathogen (Ahmed et al., 2018). The detection limit of the established LAMP assay was assessed to validate its sensitivity in the presence of pathogen DNA. In this study, the *Pectobacterium* LAMP assay detected pathogen genomic DNA at concentrations as low as 5 fg. LAMP sensitivity varies among pathogens, potentially because of bacterial traits such as extracellular polysaccharide production. Polysaccharides can inhibit DNA amplification (Schrader et al., 2012). Another study suggested that differences in sensitivity may also result from variation in primer-annealing efficiency (Lang, 2014). Shi et al. (2020) reported a LAMP detection limit of 10 pg for *P. carotovorum*, whereas Domingo et al. (2021) reported a limit of 100 fg for *P. parmentieri*.

Nucleic acid amplification is a common approach for rapid microbial detection. In contrast to polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP) is a straightforward nucleic acid amplification technique that can be performed at a constant temperature and produces a colorimetric result. LAMP assays are typically performed at 65 °C for 35–40 min (Wang et al., 2023; Zhang et al., 2019). In the laboratory, we used a water bath set to 65 °C to incubate the colorimetric LAMP reaction instead of a costly PCR thermal cycler. A major challenge in adapting LAMP assays from pure cultures to field diagnostics is the presence of complex plant-derived inhibitors. For example, Dobhal et al. (2018) developed a LAMP assay for detecting *Xanthomonas* in infected tomatoes, but their protocol was limited by plant inhibitors. In contrast, our LAMP assay was robust to such inhibition. Using a simplified sample preparation method with Tris and EDTA (TE) buffer, we avoided inhibitory effects from plant material without requiring complex DNA extraction. This result highlights the tolerance and practical field applicability of our protocol compared with existing methods. The rapid TE-buffer sample preparation developed in this study reduced sample preparation time to less than 1 min. In addition, DNA extraction was not required because plant inhibitors did not affect amplification. Other studies have used colorimetric chemistry, lateral-flow devices, and portable devices for qPCR and LAMP (Yasuhara-Bell et al., 2016; Ash, 2014). A deployable field-detection device has also been developed for olive quick decline syndrome caused by *Xylella fastidiosa*, but its use requires costly, sophisticated instrumentation and trained personnel (Ouyang et al, 2013). We used a commercially available, cost-effective, field-deployable thermal flask that is easy to operate without sophisticated instrumentation or specialized training. The current LAMP assay therefore provides rapid and reliable detection of *Pectobacterium* sp. from purified bacterial DNA and infected plant material using a portable thermal flask.

Conclusion

We developed a LAMP assay for *Pectobacterium* sp. detection that offers advantages in speed, sensitivity, specificity, simple visual detection, and minimal equipment requirements. Sample preparation for LAMP can be completed in less than 1 min, and the assay does not require purified DNA extraction. The assay provides a simple, portable, field-deployable system for specific detection of *Pectobacterium* sp. It has potential applications in routine point-of-care diagnostics, surveillance, quarantine and disease management, seed certification, and border protection.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Author contributions

HT: Data curation, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. FA: Writing – original draft, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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