Molecular and Cellular Probes 26 (2012) 175–176

Contents lists available at SciVerse ScienceDirect

Molecular and Cellular Probes

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

Short communication

Rapid on-site detection of *Acidovorax citrulli* by cross-priming amplification

Jing Zhang [a], Qian Tian [b], Shui-fang Zhu [b], Wen-jun Zhao [b,*], Feng-quan Liu [a,**]

[a] College of Plant Protection, Nanjing Agricultural University, China
[b] Chinese Academy of Inspection and Quarantine, Huixinli 241, Chaoyang District, Beijing 100029, China

ARTICLE INFO

Article history:
Received 29 January 2012
Received in revised form
29 March 2012
Accepted 29 March 2012
Available online 7 April 2012

Keywords:
Acidovorax citrulli
Cross-priming amplification
Detection

ABSTRACT

Cross-priming amplification (CPA) for *Acidovorax citrulli* detection was evaluated in this study. The sensitivity of CPA assay for pure bacterial culture was 3.7×10^3 CFU/ml. Bacteria on naturally infected watermelon seeds were detected using CPA assay, suggesting this method is suitable for *A. citrulli* on-site detection from watermelon seeds.

Acidovorax citrulli is the causative agent of bacterial fruit blotch (BFB) of watermelon [1]. Due to its economic importance, *A. citrulli* is regarded as a bacterium with plant quarantine significance in U.S., China and Europe. Current available detection methods, such as dilution plating on semi-selective media, seedling grow-out, direct PCR, real-time fluorescent PCR [2,3], have their limitations in that these methods either are time-consuming or require specific equipment. Hence, rapid, sensitive and affordable diagnostic method suitable for on-site detection is urgently needed.

Cross-priming amplification (CPA) is an isothermal DNA amplification system developed by Ustar Biotechnologies Co., Ltd., China. CPA is a class of isothermal amplification reactions that is carried out by a strand displacement DNA polymerase and does not require an initial denaturation step or addition of a nicking enzyme [4]. The detection of amplified products is visualized on a lateral flow strip housed in an enclosed, sealed plastic device to prevent the leakage of amplicons [5]. Visible bands on the test strips indicate positive reactions (Fig. 1). In this study, a CPA isothermal amplification and detection kit (Ustar Biotech, Hangzhou, China) was evaluated as whether it could accurately detect *A. citrulli* from pure bacterial culture and bacterial extracts from contaminated (or naturally infected) watermelon seeds.

A set of five primers were designed based on 16S rDNA of *A. citrulli* (GenBank AY702093.1). The five primer sequences are as follows: ACLF3: GGCTAACTACGTGCCAGC, ACLB3: ACGCATTT CACTGCTACA, ACLBIP: CAGATGTGAAATCCCCGGGCTCTGCCG TACTCCAGCGAT, ACDF5b1: BIOTIN-GCAAGCGTTAATCGGAATTACT, ACDF5f2: FITC-CAACCTGGGAACTGCATTTGT.

Sensitivity of CPA assay on *A. citrulli* was determined by analyzing product produced from a ten-fold serial dilution of *A. citrulli* pure culture (Fig. 1(A)). Our results showed that the detection limit for the kit was about 3.7×10^3 CFU/ml per reaction (equivalent to 7.4 bacterium per reaction), which is similar to the sensitivity of normal PCR assay (Fig. 1(B)).

The specificity of the CPA assay was then evaluated by detecting 18 *A. citrulli* strains and 22 reference strains from the genus of *Xanthomonas*, *Pseudomonas*, *Erwinia* and *Clavibacter*. DNA of pure bacterial cultures was isolated using TIANamp Bacteria DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. Positive results were obtained for all *A. citrulli* strains, but not for all other non-*Acidovorax* strains. However, positive results were also obtained for *Acidovorax avenae* subsp. *avenae* and *A. avenae* subsp. *cattleyae* strains. Though the CPA assay could not differentiate closely-related *Acidovorax* strains from each other due to highly conserved 16S rDNA sequence, the CPA assay could still be used for detection of *A. citrulli*, considering that *A. citrulli* is only limited to plants in the Cucurbitaceae family, such as watermelon, melon, and cucumber; whereas other *Acidovorax* strains are not linked to these plant species. Combined with host specificity and pathogenicity, the CPA assay could be useful for detection of *A. citrulli* from watermelon seeds.

Next, 12 batches of watermelon seeds naturally infected with *A. citrulli* from fields and 5 batches of healthy watermelon seeds

* Corresponding author. Tel./fax: +86 10 64912743.
** Corresponding author. Tel./fax: +86 25 84395268.
E-mail address: wenjunzhao@188.com (W.-j. Zhao).

0890-8508/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.mcp.2012.03.010

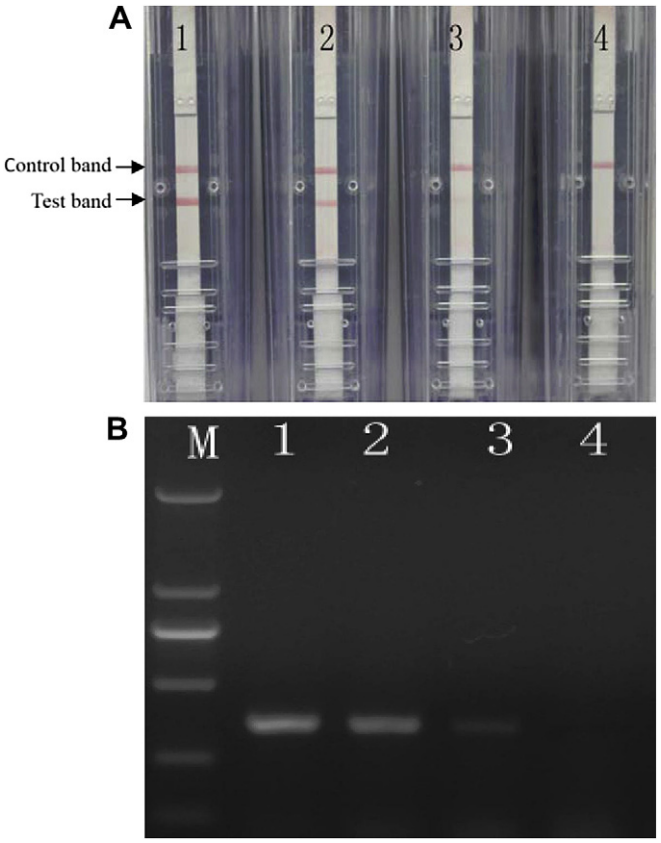

Fig. 1. (A) Sensitivity of CPA and PCR agarose gel electrophoresis detection of *Acidovorax citrulli* in pure culture. 1, 3.7×10^5 CFU/ml, 2, 3.7×10^4 CFU/ml, 3, 3.7×10^3 CFU/ml, 4, blank control. (B) M: DNA marker, 1, 3.7×10^5 CFU/ml, 2, 3.7×10^4 CFU/ml, 3, 3.7×10^3 CFU/ml, 4, blank control.

were tested by CPA and normal PCR. Seeds were suspended in sterile distilled water for 30 min and 1 ml leachate was centrifuged at 12,000 g for 10 min. DNA was extracted from precipitants using the DNA secure Plant Kit (Tiangen Biotech, Beijing, China). Positive results were obtained from all infected seeds, but not from healthy seeds, which were similar to the results from normal PCR, suggesting the CPA assay can be applied to detect bacteria from contaminated seeds.

CPA assay has been reported to detect *Mycobacterium tuberculosis* and *Enterobacter sakazakii* [6,7]. However, application of CPA in detecting plant pathogenic bacteria has not been reported. Here, we described the use of CPA kit for detection of *A. citrulli*. In this assay, DNA can be amplified by *Bst* DNA polymerase under isothermal conditions. The amplicons is detected in a simple, self-contained disposable DNA strip without opening the amplification tube, so post amplification contamination, which is associated with many nucleic acid amplification-based platforms, can be effectively eliminated. In the entire procedure, from sample preparation to visual readout, only a portable battery powered heater and small centrifuge are needed. Thus, the major advantages of this assay are its shorter turn-around time, no need of specific equipment, and easy to interpret results. So this assay can be used as an alternative to conventional PCR.

In summary, our results suggest that the CPA assay, combined the advantage of isothermal amplification with simple-to-perform and self-contained amplicons detection technology, is a valuable alternative to immunoassays and PCR-based tests for diagnosis of *A. citrulli*.

Acknowledgments

This work is financially supported by the special fund for inspection and quarantine scientific Research in the Public Interest (200810632, 201010256, 2012IK294), and the special Fund for Agroscientific Research in the Public Interest (201003066).

References

[1] Schaad NW, Postnikova E, Randhawa P. Emergence of *Acidovorax avenae* subsp *citrulli* as a crop threatening disease of watermelon and melon. *Pseudomonas syringae* and related pathogens. Biology and Genetics; 2003. p. 573–581.
[2] Minsavage GV, Hoover RJ, Kucharek TA, Stall RE. Detection of the watermelon fruit blotch pathogen on seeds with the polymerase chain reaction. Phytopathology 1995;85:1162.
[3] Weller SA, Stead DE. Detection of root mat associated *Agrobacterium* strains from plant material and other sample types by post-enrichment TaqMan PCR. Journal of Applied Microbiology 2002;92:118–26.
[4] Xu G, Hu L, Zhong H, Wang H, Yusa S, Tristen C, et al. Cross priming amplification: mechanism and optimization for isothermal DNA amplification. Scientific Report 2012;2:246.
[5] Kong H, Ranalli T, Lemieux B. New isothermal molecular diagnostics platforms. IVD Technology 2007;13:35–43.
[6] Fang R, Li X, Hu L, You Q, Li J, Wu J, et al. Cross-priming amplification for rapid detection of *Mycobacterium tuberculosis* in sputum specimens. Journal of Clinical Microbiology 2009;47:845–7.
[7] Zhao Y, Zhang X, Zhang H, Liu W, Zheng W, Huang X. Rapid and sensitive detection of *Enterobacter sakazakii* by cross-priming amplification combined with immuno-blotting analysis. Molecular and Cellular Probes 2010;24:396–400.