Assessment of new biomaterials for sample collection and nucleic acid recovery

D.J. Caasi, M. Arif and F.M. Ochoa-Corona
Oklahoma State University, Department of Entomology and Plant Pathology, National Institute for Microbial Forensics and Food & Agricultural Biosecurity (NIMFFAB), Stillwater, OK, U.S.A. 74078
E-mail: francisco.ochoa_corona@okstate.edu and donna.caasi@okstate.edu

INTRODUCTION

- Paper-based technologies facilitate the storage of nucleic acids (NA) at room temperature; however, the recovery of NA from these types of technologies requires extraction steps and streamlining PCR is hampered by residues such as chemical components and the paper matrix (fiber) itself.
- Collecting and archiving NA are key steps during detection and diagnosis when using PCR for agricultural biosecurity or microbial forensics applications.

OBJECTIVES

- To characterize new biomaterials and test their effectiveness in sample collection and nucleic acid recovery for PCR amplification.

MATERIALS AND METHODS

- 5 Biomaterials, varying compositions (Manufacturer’s confidentiality) and thicknesses:
  - 1 = 50 µm
  - 2 = 83 µm
  - 3 = 76 µm
  - 4 = 50 µm
  - 5 = 173 µm

- Wet & dry biomaterials, with and without bacteria (Pseudomonas syringae pv. tomato)

- 1.2 mm disc core in 10 µL, 15 µL, 20 µL, and 50 µL of water

- Pore spaces and crevices, Scanning Electron Microscope (FEI Quanta 600F)

- Residues/absorbance (O.D.260 and 280 nm) (Nanodrop nd-1000)

- Sample collection:
  - 1 µL of DNA from Pythium spinosum or dsRNA from Citrus Leprosis Virus C sample blotted into biomaterial

- Nucleic acid recovery:
  - 1.2 mm disc core of biomaterial (NA-blotted) taken

- Directly added to PCR mix

- PCR (DNA) RT-PCR (dsRNA)

LAB PROTOCOLS

1. PCR amplifications were carried out in 20 µL volume containing 10 µL GoTag Master Mix (2x), 1 µL of forward and reverse primers (5 µM) each, 8 µL nuclease-free water, and 1 µL of DNA sample or DNA from 1 disc core. PCR cycling conditions were 94°C (initial denaturation) for 3 min., 40 cycles at 94°C (denaturation) for 20 sec., 62°C for 15 sec. (annealing), and 72°C for 20 sec (extension); final extension was at 72°C for 3 min. Product size = 349 bp.

2. RT-PCR amplification was carried out in 20 µL volume containing 10 µL reaction mix (2x), 1 µL RNaseOut, 2 µL of forward and reverse primer (5 µM) each, 0.8 µL SSIII/Platinum Taq, 1 µL BSA (10 µg/µL), 2.2 µL nuclease-free water, and 1 µL of RNA sample or 1 disc core with RNA. PCR cycling conditions were 50°C for 30 min for cDNA synthesis, 94°C for 2 min. (initial denaturation), 40 cycles at 94°C for 30 sec. (denaturation), 55°C for 30 sec. (annealing), and 72°C for 30 sec. (extension); final extension was at 72°C for 7 min. Product size = 278 bp.

RESULTS AND DISCUSSION

- Pore spaces and crevices were retained in biomaterials after wetting (Fig. 1A and 1B). Microorganisms are retained within matrix spaces, see bacteria (Fig. 1C and 1D).

- Absorbance of residues from biomaterials ranged between 0.014 and 0.034 O.D.260 and from 0.013 to 0.030 O.D.280. Residues were higher in the thickest biomaterial (173 µm) and lowest in the thinnest (50 µm). The presence of residues in biomaterials can affect the measurement of NA concentrations and might interfere with PCR reactions (Figure 2).

- Detected residues did not interfere with PCR amplification of P. spinosum DNA and RT-PCR of Citrus Leprosis Virus C dsRNA (Figure 3).

CONCLUSION AND RECOMMENDATIONS

1. All new biomaterials tested retain their pore spaces and crevices even after wetting. The biomaterials contain residues that are detectable at 260 nm and 280 nm wavelengths. Caution should be observed when measuring concentration of NA stored in biomaterials since both NA and residues absorb at the same wavelengths.

2. The characterized biomaterials can be applied for streamlining both PCR-based amplification and NA storage, and have potential applications in collection and recovery of microbial NA.

Figure 1. Scanning electron micrograph of a representative biomaterial (83 µm thick) in dry (A) and wet (B) forms without bacteria, and wet with bacteria (Pseudomonas syringae pv. tomato) (C) and (D).

Figure 2. Absorbance of residues from biomaterials of varying thicknesses at 260 and 280 nm. Biomaterial thicknesses are: 1=50 µm, 2=83 µm, 3=76 µm, 4=50 µm, and 5=173 µm. Absorbance with the same letter of the same wavelength are not significantly different at 5% level of significance.

Figure 3. PCR amplification in five biomaterials: A) different dilutions of DNA from Pythium spinosum, and B) cDNA synthesized from dsRNA of Citrus Leprosis Virus C. DNA or dsRNA were blotted into five biomaterials of varying thicknesses as follows: 1=50 µm, 2=83 µm, 3=76 µm, 4=50 µm, and 5=173 µm. The NA positive control (+), consisted of a NA solution and was not blotted into biomaterials.