VERSION: 1.0	**DATE:** 12/2012
PATHOGEN: Curtobacterium flaccumfaciens pv. flaccumfaciens (syn: Corynebacterium flaccumfaciens pv flaccumfaciens)	
HOST: common bean (Phaseolus vulgaris	
COMMON NAME: bacterial wilt	
METHOD: Be 4.1 PCR Assay (Tegli et al., 2002)	
METHOD CLASS: TEMPORARY STANDARD (B)	
SAMPLE: 15,000	

PROCEDURE:

The authors used three subsamples of 5000 seeds each, and claim the detection limit with this method is as low as 10^2 CFU/ml, while the threshold was 10^3 CFU/ml if the DNA was extracted with the Puragene DNA Isolation Kit (Gentra).

Seed wash

1. To create a positive sample add 1ml of CFF cell suspension to a sample of healthy seeds.

2. Soak seeds in one liter of solution (0.85% NaCl in distilled water) for approximately 12 h at 4°C while shaking at 100 rpm.

3. Filter supernatant through cheesecloth.

4. Concentrate supernatant by centrifugation (9000g at 4°C for 30 min).

5. Resuspend pellet in 3 ml of sterile physiological solution (0.85% NaCl in distilled water).

6. Plate aliquots (100 ul plate) on NBY to assess the number of viable CFF cells, estimated as CFU/ml. Note you may need to do a 10-fold dilution.

7. Centrifuge two subsamples of 1ml each and use these pellets for bacterial DNA extraction.

DNA extraction

1. To extract bacterial DNA use a Puragene DNA Isolation Kit (Gentra System Inc., Minneapolis, MN, USA) and the Instagene Matrix (Bio-Rad, Hercules, CA, USA), and follow the manufacturers' instructions.

PCR amplification

The authors used the primer pair ERIC1R-ERIC2 (Versalovic et al., 1994) and an automated thermal cycler (Delphy 1000TM, Oracle BiosystemsTM, MJ Research Inc., Watertown, MA, USA).

1. Carry out reactions in a total volume of 25ul.

2. The reaction mixture should contain approximately 50ng of DNA template, 20mM Tris-HCl (pH 8), 50mM KCl, 6.7 mM $MgCl_2$, 625uM of each of the four deoxynucleotide triphosphates (dNTPs: dATP, dCTP, dGTP, dTTP), 2uM of each primer and 2 U Taq DNA polymerase (Polymed s.r.l., Florence, Italy).

3. PCR program: an initial denaturation at 95°C for 7 min, 30 cycles of denaturation (1 min at 94°C), primer annealing (1 min at 52°C) and primer extension (8 min at 65 °C), followed by a final extension at 65°C for 16 min.

4. Use electrophoresis to analyze the PCR product (306bp product).

REFERENCES:

Tegli, S., Sereni, A. and Surico, G. 2002. PCR-based assay for the detection of Curtobacterium flaccumfaciens pv. flaccumfaciens in bean seeds. Letters in Applied Microbiology. 35(4):331-337. Versalovic, J., Schneider, M., de Bruijn, F. J. and Lupski, J. R. 1994. Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Methods in Molecular and Cellular Biology. 5:472–489.

Mohan S. K. and Schaad N. W. 1987. An improved agar plating assay for detecting Pseudomonas syringae pv. syringae and P. s. pv. phaseolicola in contaminated bean seed. Phytopathology. 77(10):1390-1395 Sands D. C., Schroth, M. N. and Hildebrand, D. C. 1980. Pseudomonas. Pages 36-44 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. NW Schaad (ed.) American Phytopathological Society, St. Paul MN. 72p.