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Rapid diagnostic assay for detection of plant pathogenic bacteria using colloidal gold nanoparticles.

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Abstract

Sensitive methods for rapid, selective detection of plant pathogens are very desirable for effective diagnostic in plant production. Appropriate detection methods should be simultaneously sensitive and specific as well as simple, fast, reliable and reproducible. The one of the fastest growing trends of the research in this area is the use of nanoparticles in diagnostic assays. The antibody-conjugated nanoparticles can readily and specifically identify a variety of bacterium, even form diagnostically difficult samples.

The presented solution based on detection of quarantine bacterium Clavibacter michiganensis subsp. sepedonicus (Cms)as a model relies on the immunological bacterial cells staining via conjugate of colloidal gold nanoparticles and the antibodies specific to the pathogen. The gold colloid was prepared by reduction of gold ions. Gold colloid was fractionated by centrifugation and standardized using UV-Vis spectroscopy. In order to produce a sensitive and specific marker for the optical detection of Cms bacteria, colloidal gold nanoparticles were coated with IgG anti-Cms both in oriented and random method. The Cms bacteria cells thermally fixed on the surface of the microscope slides were stained with both of the colloidal gold –IgG conjugates. In the next step to amplify the optical signal, silver ions were reduced on coated gold nanoparticles. As a reference probes for bacterial cells staining, commercially available anti Cms-IgG – Alkaline phosphatase enzyme and anti-Cms – IgG FITC conjugates were used. In the first case the enzymatic assay was observed using classical optic microscope, On the other side the studies were carried out by fluorescence microscope using standard indirect immunofluorescent antibody assay (IF).

Compared to the IFAS method, the colloidal gold nanoparticles based assay was cheaper, faster, more sensitive and not required to use of fluorescence microscope. The additional step using reduction of silver ions on colloidal gold nanoparticles improved optical signal and sensitivity of the gold based assay. On the other hand, the enzymatic assay giving insoluble product gave optical signal but the background was relatively high.