APPLICATION OF SOME NANOTECHNOLOGICAL APPROACHES IN RAPID DETECTION OF FEED-BORNE PATHOGENIC BACTERIA

By

ISLAM MOHAMED TORK GABER MOSTAFA
B. Sc. Agric. Sci. (Desert Land Reclamation and Cultivation), Fac. Agric., Cairo Univ., 2008

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APPROVAL SHEET

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ABSTRACT

This study was planned to exploit the most recently innovated noble nanoparticles in a highly sensitive colorimetric real-time-assay for detecting *E. coli* O157:H7 in feedstuffs. Gold nanoparticles (AuNPs) were prepared adopting the citrate method and the obtained colloidal nanoparticle suspension was characterized using the UV–vis spectroscopy, Dynamic light scattering (DLS), Zeta potential instrument, Transmission Electron Microscopy (TEM) and X'Pert PRO X-ray Diffraction System (XRD). Results showed that the prepared AuNPs were well dispersed colloidal spherical shaped nanosized particles averaged 40 nm in size having a characteristic surface plasmon resonance band similar to gold nanoparticles and absorbance peak of 530 nm. Zeta potential of the prepared AuNPs verified the negatively charged surfaces of -32 mv. Specific *E. coli* O157:H7 antiserum was conjugated to the surfaces of the prepared AuNPs and the developed AuNPs-conjugated suspension had a red color. Stability of the AuNPs- *E. coli* O157:H7 antiserum conjugate suspension was found to be greatly affected by the antibody concentration used in the conjugation mixture. A maximum antibody concentration of 50 µl ml^{-1} is required for permanent stability of the conjugate. Higher antibody concentrations than 50 µl ml^{-1} resulted in unstable, flocculated or completely aggregated conjugate suspensions. Conjugated AuNPs *E. coli* O157:H7- antiserum suspension was used for detecting *E. coli* O157:H7 in yellow corn sample contaminated with this bacterium. All examined serial dilutions of the contaminated yellow corn gave blue color after mixing with 100 µl aliquots of the AuNPs antibody conjugate while the sterilized yellow corn retained the red color of the conjugate. To examine the specificity of the prepared AuNPs –antibody conjugate, yellow corn contaminated with *Salmonella typhimurium*, *Staphylococcus aureus* or *Bacillus cereus* were tested using the AuNPs – antibody conjugate where no color changes were observed after mixing with the conjugate. Microbiological analysis of pointed out to a limited microbial load in the eight feedstuffs namely fish meal, protein concentrate, yellow corn, soybean meal, corn gluten, rabbit feed and ruminant feed as the total viable count ranged between 15×10^3 and 3×10^4 CFU g^{-1}. Conventional microbiological analyses for the presence of *E. coli* O157: H7 pointed out to the absence of such bacterium in all samples except the fish meal and protein concentrate. These feed samples were examined for the occurrence of *E. coli* O157: H7 using the prepared AuNPs-antibody conjugate. The results emphasized the occurrence of *E. coli* O157: H7 in both samples. The obtained results suggested further investigation on the possible application of the prepared AuNPs- *E. coli* O157: H7-antiserum in a biosensor for detection of *E. coli* O157: H7 in feedstuffs.

Key words: *E. coli O157:H7* Detection, Antibody, Colloidal gold nanoparticles, Conjugation and Colorimetric.

DEDICATION

This work is dedicated to whom my heartfelt thanks; to my mother, my father, my husband and my sisters for their patience, help and for all the support they lovely offered during my post graduation studies.

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