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Detection of environmentally hazardous pesticide in fruit and vegetable samples using gold nanoparticles

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ABSTRACT

A new approach for simple, competitive and sensitive, dipstick immunoassay (DIA) was developed to detect organophosphorus pesticide. Residual pesticides such as kitazine in fruits and vegetables are one of the major food safety concerns for consumers in the world. It is the alternative method to nitrocellulose strip (NC) for the detection of harmful pesticides. The matrix effects of different food samples (tomato, cucumber, grapes and orange) were studied in flow through ELISA (Enzyme Linked Immunoassay) and DIA method. Different size morphology was synthesized from gold nanoparticles and bioconjugated to Ig G, which served as the detecting agent confirmed by AFM. The different concentration of organophosphorus in the food sample was detected by the development of purple color in the membrane strips. Among four fruit juices, the grape juice exhibits increased absorbance resulted that the bioconjugated gold nanoparticle is actively detected the kitazine present in the grape juice evidenced by ELISA method has good reproducibility and satisfactory accuracy. The interpretation of the results was visual, and these ELISAs can be used as convenient qualitative tools for the rapid screening ofkitazine residues in food samples.

1. Introduction

Owing to their extraordinary physical and chemical properties, gold nanoparticles play a vital role in nanotechnology such as spectroscopic, pesticide detection, colorimetric and electrochemical methods. Different analytical methods can be used to detect pesticides with high sensitive analysis of agriculture chemicals in fruits and vegetables, but they require being highly trained instrument operators using expensive equipment (Pang et al., 2006). Accordingly, there is an impetus to develop an immunoassay for detecting pesticides, with an aim of simplifying sample preparation, without technical expertise and analytical equipment. Currently, DIA is used bioconjugated gold as reporters for pesticide detection which require no special equipment and solvents, and the visible results of DIA can decide by the onsite.

In recently, ELISA has been used to detection of pesticides in fruits and vegetables, and shows great assure as a sensitive and rapid detection method (Fan, Lai, Rasco, & Huang, 2014; Fan, Lai, Rasco, & Huang, 2015; Liu et al., 2012, 2013). The one of the hottest areas is DIA and ELISA application in detection of a pesticide in the skins of fruits and vegetables. The compound such as organophosphorus, bioconjugated gold nanoparticles and DIA has good selective detection. These belongings to make them attractive as a potential application and probes for multi-pesticides analysis. Different specific antibodies against organophosphorus have been developed for enzyme-linked immunoassay (ELISA). While several methods can be used to develop the pesticide detection, they are preferred ELISA method is high sensitivity and reproducibility. The major application of ELISA method is the detection of pesticides, based on enzyme inhibition (Meulenberg, De Vree, and Dogterom, 1999).

Immunoglobulin G (IgG) is a type of antibody. It is protein complex composed of four peptide chains — two identical heavy chains and two identical light chains arranged in a Y — shape typical of monoclonal antibody in a human being.

Kitazine, a new fungicide widely used on fruits and vegetables in exerts systemic fungicidal action and possesses some insecticide properties (Anon et al., 1988). The wide spread of kitazine as crop

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protectant in agriculture and animals with this compound during various farm operations was considered for testing the compound for its irritancy property. As pesticides are hazardous and toxic to human health, any pesticide residue remaining in fruits and vegetables can pose danger to humans and cause certain diseases. It is important to identify and quantify the pesticides which can be ingested by fruits and vegetables after pesticide spray. The previous reports have shown the practicability of applying DDT for detection of environmental samples contains gas chromatography electron capture detection (GCECD), HPLC with UV detection and GC-mass spectrometry (Tomiyama et al., 2000; Moriwaiki and Mivakoda 2007).

The main objectives of the present study were to investigate novel bioconjugated gold nanoparticles to develop a fast, well founded and economical methods for quantifying trace amounts of kitazine.

2. Materials and methods

2.1. Isolation and identification

The sulfur reducing bacteria were isolated from chemical company effluent collected from Vellore. The collection of samples was inoculated in a specific mineral medium. The isolated organism was maintained for sulfur-reducing medium (Jones, Simon, & Gardener, 1982). The organism was morphologically and biochemically characterized as *Serratianematodiphila* (CAA) was mentioned in our previous studies (Malarkodi et al., 2013).

2.2. Sequencing of 16Sr-RNA gene

Genomic DNA was isolated by the method described by Murray and Thampson (1980). The integrity of the obtained genomic DNA was detected by electrophoresis in 1% agarose gel (Sigma-Aldrich, India) stained with ethidium bromide. The 16S rRNA region was amplified with forwarding 5′ GATCCTGGCTCAGGATGAAC 3′ and reverse 5′GGACTACCAGGGTATCTAATC 3′ bacterial universal primer pairs. The reaction mixture for PCR amplification was prepared in a total volume of 50 μl with 10 x PCR buffer, 10 mM of dNTPs, 1.0 μl of the Taq polymerase and 200 pmol of forward and reverse primers. The PCR reaction details were as follows: 5 min at 95 °C for initial denaturation, 1 min at 95 °C for denaturation, 2 min at 72 °C for the final extension. The 16S rRNA JQ701743 (CAA) was purified using GenElute TM Gel Extraction Kit (Sigma-Aldrich, USA) and sequencing of 16S rRNA gene was done in an automated ABI-3100 Genetic Analyser (GeNei, India).

2.3. Phylogenetic analysis

The existing sequence from GenBank with of representative species was used for phylogenetic analyses. MEGA5 (Tamura, Peterson, Peterson, Stecher, & Nei, 2011) software program was started with a set of aligned sequences using MUSCLE, and searches for phylogenetic trees that are optimal according to NJ algorithm under p-distance model. The bootstrap value was set as 1000 replication. The GenBank accession number of sequence reported in this paper is JQ701743 (CAA). The genetic similarity between the sequences was identified by using BODIL (Lehtonen et al., 2004) under the malign program (Johnson & Overington, 1993).

2.4. Synthesis of gold nanoparticles

The *S. nematodiphila* (CAA) were grown up in a conical flask containing 100 ml of nutrient broth in a shaker incubator at 35 °C. After 24 h incubation, biomass developed on the medium. Auric Chloride (HAuCl4) was added into the culture medium. Then the reaction mixture was left for a further 24–72 h in a shaker incubator at 35 °C. Biotransformation took place after incubation i.e., chloroauric ions were reduced to gold nanoparticles. The accumulation and reduction of gold were examined by visual observation of the medium. The medium turned pale yellow to brick red color which was the clear indication of the formation of gold nanoparticles.

2.5. Preparation and characterization

The synthesis of gold ions in aqueous solution was monitored by UV–vis spectrophotometer of the solution between 300 and 600 nm using Perkin – Elmer spectrophotometer. The nanoparticles powder was prepared using centrifugation and washing by milliq water. The nanoparticles containing medium was centrifuged at 12320 g for 15 min and collected pellet was washed with milliq water. Again it was centrifuged and pellet was collected for drying at 80 °C and the collected powder was used for characterization process. The crystalline phases of the products were determined by X-ray powder Diffractometer (Seifert – 3000p). The SEM micrograph of the sample was recorded using and JEOL 6610 LV. TEM was obtained using FEI Technai G2 20 electron microscope operating at 200 kV. The nanoparticles were scanned the infrared in the region of 4000–400 cm^{-1} Fourier Transform Infrared spectrometer (Thermo Nicolet Model – 6700). AFM micrographs were recorded in the tapping mode with a Nanoscope III (Digital Instruments, model MMAFM-2, scan stage J). The ELISA was carried out in 96 well microplates (Xiamen Yunpeng Technology Development Co., LTD., Xiamen, China). Absorbance was measured at a wavelength of 450 nm using Multiskan MK3 microplate reader (ThermoLabsystem).

2.6. Conjugation of gold nanoparticle

The antibody (IgG) (*Staphylococcus aureus* antibody (702) SC 58306) purchased from Santa Cruz Biotechnology, Inc. The bacteria *S. nematodiphila* synthesized gold nanoparticle was conjugated with the antibody (IgG)(*Staphylococcus aureus* antibody (702) SC 58306) by adding 20 ml of colloidal gold solution (pH 8.0) was mixed with 400 μg of antibody (IgG). (IgG) antibodies were added drop by drop with gentle stirring. After overnight incubation at 4 °C, the solution was centrifuged at 12320 g for 30 min at 4 °C. The pellet was resuspended in the minimum quantity of conjugate storage buffer (1 mM phosphate buffer of pH 7.0 with 0.05% Tween-20). The binding of antibodies to Gold nanoparticles was confirmed by the absorption of UV– Vis spectrum analysis of the pure gold nanoparticles and antibody conjugated gold nanoparticles in the wavelength ranges from 400 to 750 nm.

2.7. Kitazine detection of NCmembrane strip

Nitrocellulose (NC) membrane strip (pore size = 0.22 μm, 13 mm in diameter) was provided by Sigma-Aldrich. Different concentrations of kitazine 0.1 μl, 0.3 μl 0.5 μl, 0.7 μl and 1 μl were taken in container and makeup total volume to 1 ml with (IgG) BGNPs. Nitrocellulose membrane strip was dipped in various dilutions of BGNPs. The test strips were dried at 35 °C for 30 min. The development of intense purple color was monitored which served as a control without addition of kitazine.

2.8. Food sample detection

2.8.1. Preparation of spiked samples

Mango, tomato, grapes, cucumber and orange were bought from