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Gold Nanoprobes Assay for the Identification of Foodborn Pathogens Such as Staphylococcus aureus, Listeria monocytogenes and Salmonella enteritis

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Abstract : Objectives: Nanotechnology is providing revolutionary opportunities for the rapid and simple diagnosis of many infectious diseases. Staphylococcus aureus, Listeria monocytogenes and Salmonella enteritis are important human pathogens. Diagnostic assays for bacterial culture and identification are time consuming and laborious. There is an urgent need to develop rapid, sensitive, and inexpensive diagnostic tests. In this study, a gold nanoprobe strategy developed and relies on the colorimetric differentiation of specific DNA sequences based approach on differential aggregation profiles in the presence or absence of specific target hybridization. Method: Gold nanoparticles (AuNPs) were purchased from Nanopartz. They were conjugated with thiolated oligonucleotides specific for the femA gene for the identification of members of Staphylococcus aureus, the mecA gene for the differentiation of Staphylococcus aureus and MRSA Staphylococcus aureus, hly gene encoding the pore-forming cytolysin listeriolysin for the identification of Listeria monocytogenes and the invA sequence for the identification of Salmonella enteritis. DNA isolation from Staphylococcus aureus Listeria monocytogenes and Salmonella enteritis cultures was performed using the commercial kit Nucleospin Tissue (Macherey Nagel). Specifically 20µl of DNA was diluted in 10mMPBS (pH5). After the denaturation of 10min, 20µl of AuNPs was added followed by the annealing step at 58oC. The presence of a complementary target prevents aggregation with the addition of acid and the solution remains pink, whereas in the opposite event it turns to purple. The color could be detected visually and it was confirmed with an absorption spectrum. Results: Specifically, 0.123 µg/µl DNA of St. aureus, L.monocytogenes and Salmonella enteritis was serially diluted from 1:10 to 1:100. Blanks containing PBS buffer instead of DNA were used. The application of the proposed method on isolated bacteria produced positive results with all the species of St. aureus and L. monocytogenes and Salmonella enteritis using the femA, mecA, hly and invA genes respectively. The minimum detection limit of the assay was defined at 0.2 ng/µL of DNA. Below of 0.2 ng/µL of bacterial DNA the solution turned purple after addition of HCl, defining the minimum detection limit of the assay. None of the blank samples was positive. The specificity was 100%. The application of the proposed method produced exactly the same results every time (n = 4) the evaluation was repeated (100% repeatability) using the femA, hly and invA genes. Using the gene mecA for the differentiation of Staphylococcus aureus and MRSA Staphylococcus aureus the method had a repeatability 50%. Conclusion: The proposed method could be used as a highly specific and sensitive screening tool for the detection and differentiation of Staphylococcus aureus Listeria monocytogenes and Salmonella enteritis. The use AuNPs for the colorimetric detection of DNA targets represents an inexpensive and easy-to-perform alternative to common molecular assays. The technology described here, may develop into a platform that could accommodate detection of many bacterial species.

Keywords : gold nanoparticles, pathogens, nanotechnology, bacteria
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