A simple but promising electrochemical DNA nanosensor was designed, constructed and applied to differentiate a few food-borne pathogens. The DNA probe was initially designed to have a complementary region in Vibrio parahaemolyticus (VP) genome and to make different hybridization patterns with other selected pathogens. The sensor was based on a screen printed carbon electrode (SPCE) modified with polylactide-stabilized gold nanoparticles (PLA-AuNPs) and methylene blue (MB) was employed as the redox indicator binding better to single-stranded DNA. The immobilization and hybridization events were assessed using differential pulse voltammetry (DPV). The fabricated biosensor was able to specifically distinguish complementary, non-complementary and mismatched oligonucleotides. DNA was measured in the range of $2.0 \times 10^{-9}$–$2.0 \times 10^{-13}$ M with a detection limit of $5.3 \times 10^{-12}$ M. The relative standard deviation for 6 replications of DPV measurement of 0.2 µM complementary DNA was 4.88%. The fabricated DNA biosensor was considered stable and portable as indicated by a recovery of more than 80% after a storage period of 6 months at 4–45 °C. Cross-reactivity studies against various food-borne pathogens showed a reliably sensitive detection of VP.

**Keyword**: Electrochemical DNA biosensor; Polylactide-stabilized gold nanoparticles (PLA-AuNPs); Nucleic acid hybridization detection; Methylene blue; Food-borne pathogens