Electrochemical DNA biosensor for the detection of specific gene related to Trichoderma harzianum species

Abstract

A new electrochemical biosensor is described for voltammetric detection of gene sequence related to Trichoderma harzianum. The sensor involves immobilization of a 20 base single-stranded probe (ssDNA), which is complementary to a specific gene sequence related to T. harzianum on a gold electrode through specific adsorption. The DNA probe was used to determine the amount of target gene in solution using methylene blue (MB) as the electrochemical indicator. The covalently immobilized probe could selectively hybridize with the target DNA to form a hybrid on the surface despite the bases being attached to the electrode. The changes in the peak currents of methylene blue (MB), an electroactive label, were observed upon hybridization of probe with the target. Peak currents were found to increase in the following order: hybrid-modified AuE and the probe-modified AuE which localized to the affinity of MB. Control experiments with the non-complementary oligonucleotides were performed to assess whether the DNA biosensor responds selectively, via hybridization, to the target. DNA biosensor also able to detect microorganism at the species levels without nucleic acid amplification. The redox current was linearly related to the concentration of target oligonucleotide DNA, ranged from 1-20 ppm. Numerous factors, affecting the probe immobilization, target hybridization and indicator binding reactions are optimized to maximize the sensitivity and reduce the assay time.