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Entry No. 2020RDZ8706

Summary of PhD Research

Title: Design, Development, and Evaluation of a Highly Sensitive and Specific Aptamer for the Detection of Carbendazim for Food and Environmental Applications

This PhD research focused on the design, development, and evaluation of DNA aptamer-based strategies for carbendazim (CBZ) detection in food and environmental applications. The study addressed the limitations of conventional analytical methods, including HPLC, LC-MS, GC-MS, and ELISA, and the need for rapid, sensitive, cost-effective, and field-deployable detection platforms. Initially, CBZ-specific aptamer pools were developed using gold nanoparticle (GNP)-assisted high-throughput systematic evolution of ligands by exponential enrichment (HT-SELEX). Counter-SELEX and complementary colorimetric, fluorescence, and RT-PCR analyses were employed to monitor enrichment across SELEX cycles 0-12th. Next-generation sequencing (NGS) and bioinformatics tools were used to analyse sequence abundance and enrichment, identify conserved motifs, and predict aptamer secondary structures, leading to the shortlisting of 19 candidate aptamers. Computational studies subsequently investigated aptamer-CBZ interactions through ensemble molecular docking, molecular dynamics simulations, structural stability analysis, hydrogen-bond assessment, and MM-PBSA binding-energy calculations. Furthermore, a two-layer stacked random-forest machine-learning model integrating global and local aptamer features, structural information, and small-molecule descriptors was developed for SELEX-free aptamer discovery. The model achieved approximately 85% accuracy and a ROC-AUC of 0.92. SHAP-based interpretation and experimental validation of predicted candidates using a SYBR Green I fluorescence assay connected computational screening with experimental evaluation. Finally, the aptamer-based recognition strategy was translated into a lateral flow assay (LFA) for rapid, visual CBZ detection. The platform employed GNP-aptamer conjugates and a CBZ-induced aptamer-complementary DNA (cDNA) dissociation mechanism. cDNA hybridisation and dissociation were evaluated using SYBR Green I fluorescence and gel electrophoresis, followed by optimisation of the test and control lines. The developed LFA demonstrated a reported limit of detection (LOD) of 1.5 μ M for CBZ under optimum condition. Overall, this multidisciplinary research integrates experimental aptamer selection, bioinformatics, molecular modelling, machine learning, and LFA development, providing a foundation for further optimization and validation of rapid CBZ detection in real-world food and environmental samples.