Detection of The Herbicide Atrazine in Freshwater Using DNA Aptamers

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Agricultural producers across the country rely on atrazine as the foundation of their weed control programs. Widespread pollution of the environment by atrazine is a major concern as an increasing number of rivers and aquifers have been observed to be contaminated by these herbicides. Understanding the spatial extent of such contamination would be improved if a cost-effective methodology for the detection of atrazine were available. We have developed DNA aptamers which offer a reasonably-priced methodology for not only the sensitive detection, but also the bioremediation, of atrazine in California's freshwater systems.

Many crops, commodities and services in the U.S. could not be supplied in an economic fashion without the use of the herbicide atrazine in weed control programs. However, because of the extreme toxicity of atrazine, its maximum contaminant level (MCL) has been set at 3 parts per billion (ppb) by the EPA. A survey by the Cal/EPA's Department of Pesticide Regulation (DPR) of freshwater samples taken from 3,564 wells in 48 of California's 58 counties revealed the presence of atrazine above regulatory limits in many areas, especially where soil characteristics favor the movement of pesticides to groundwater.

The objective of the proposed research was to develop novel single-stranded deoxyribonucleic acid (ssDNA) aptamers for the selective and cost-effective detection and bioremediation of atrazine in drinking water supplies. ssDNA aptamers are a new class of bioreceptors that have affinity characteristics for ligands similar to those of antibodies, but that do not require either the immunization of animal hosts or mammalian cell culture. ssDNA aptamers can be selected using combinatorial selection approaches and subsequently should be able to be economically mass produced to provide a cost-effective method for large scale water treatment application.

We used Systematic Evolution of Ligands by Exponential Enrichment (SELEX), a combinatorial selection technique to select ssDNA aptamers with affinity for atrazine. A 66 bases DNA library with 30-mer random DNA flanked with priming sites and labeled with radioactive P^{32} was incubated with magnetic polystyrene beads modified with atrazine. Then, recovery/elution was accomplished using a high concentration of atrazine solution and amplification of the recovered ssDNA by polymerase chain reaction. Eleven rounds of SELEX were performed and a decreasing diversity of the ssDNA library (higher percentage of atrazine binder in the library) with each passing round was observed (0.4% to 40%).

The binding affinity constant, K_d, of the ssDNA library was determined to be 7 μM. The cloning of the library in a bacterium host followed by screening of a single ssDNA sequence is expected to provide a receptor with improved K_d suitable for atrazine monitoring and remediation applications in freshwater systems.

Collaborative Efforts

A DNA aptamer-based fluorescence polarization bioassay is being developed in conjunction with Intelligent Optical Systems, Inc., of Torrance, CA.

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