

IN VITRO SELECTION OF DNA APTAMERS AGAINST TOBRAMYCIN. Nick Coleman, Jie Zhu and Balazs Siminszky, Research Analyst, Graduate student and Professor, Agronomy Department, University of Kentucky, Lexington, KY 40546-0312.

Aptamers are small, single-stranded DNA or RNA molecules that can specifically bind to a target. Developed in the 1990s, aptamers have been soon recognized as powerful research tools due to their wide target range, high-affinity target binding, exquisite specificity, great structural stability and simple production, features that allow the selection of aptamers for most proteins and a wide range of small molecules quickly and economically. While aptamers found several practical applications in biomedical and basic biochemical research, their potentials in agrochemistry, environmental toxicology and pesticide physiology have not yet been tested. In recent years our laboratory has been involved in developing aptamers against small molecules with the ultimate goal of selecting ssDNA aptamers against pesticides. To optimize the selection process, we chose tobramycin, an aminoglycoside antibiotic, as one of our targets. Tobramycin is substituted with 5 amino groups that carry positive charges under physiological pH and facilitate the immobilization of the molecule to solid support, features that render tobramycin an ideal target for aptamer selection. We immobilized tobramycin to an agarose matrix and performed the SELEX procedure to isolate the ssDNA aptamers that specifically bind to tobramycin. After 12 selection cycles, the complexity of the ssDNA pool was reduced to a single DNA sequence that displayed affinity to tobramycin. Our future direction is to evaluate the feasibility of using the anti-tobramycin aptamers as solid-phase extraction matrices for tobramycin detection. The results of these experiments will be used to extend this technology to research in the areas of herbicide safeners and pesticide residue analysis.