Development of High-Affinity Aptamers for *Xylella fastidiosa* Detection Using Comparative SELEX Strategies

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Xylella fastidiosa is one of the most devastating plant pathogens in Europe, responsible for severe economic losses in olive, grapevine, citrus, and other high-value crops. The development of specific, sensitive, rapid, and low-cost diagnostic tools is essential for effective disease monitoring and surveillance. Aptamers, short, single-stranded nucleic acids that function as molecular antibodies, represent a promising platform for field-deployable diagnostics due to their stability, specificity, and ease of integration with diverse detection technologies.

In this study, a tentative for high-affinity aptamers targeting *Xylella fastidiosa* (*Xf*) was carried out through 15 rounds of Systematic Evolution of Ligands by Exponential Enrichment (SELEX), followed by one round of counter-SELEX using a cocktail of bacterial strains isolated from olive trees sap to eliminate non-specific binders. Three distinct SELEX strategies were employed and compared: Centrifugation-based partitioning [1], Membrane filtration [2], and a novel Antibody-assisted approach introduced for the first time in this study. Aptamers pools from round 2, 5, 10, 15 and 16 (counter-SELEX) were sequenced using Illumina next-generation sequencing and analysed with the AptaSUITE software [3].

Among the tested approaches, the Antibody-assisted SELEX was the most effective in enriching highly specific candidates, yielding 10 unique aptamers, two of which were also identified in the other two methods. The binding affinity of these aptamers to *Xf* was assessed using quantitative PCR (qPCR) and Surface Plasmon Resonance (SPR) assays. Two Aptamers confirmed their high specificity and strong interaction with *Xf*. These aptamers hold substantial potential for the development of rapid, reliable, and portable diagnostic tools for *Xf* detection, and could be further integrated with bactericidal agents for enhanced pathogen control.

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