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Biosensors for Selective and Label-free Detection of *Xylella fastidiosa*

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The *Xylella fastidiosa* bacterium is one of the most hostile pathogenic microorganisms causing multiple plant diseases, with huge economic impact on both agriculture and environment. [1] By revealing the presence of the bacterium before symptom arrival, preventive action plans could be applied to confine the contagion. In this scenario, the development of a surveillance device, conveying high sensitivity along with early detection of *X. fastidiosa* outbreaks, would be of paramount importance. Fundamental to the development of such a detection system is the availability of high binding affinity antibodies for *X. fastidiosa* (anti-XF), which could be integrated in the sensor transducers. Also, a label-free detection system would be desirable, which shorts the analysis process to achieve optimal time-to-results for pathogen identification.[2]

In this study Surface Plasmon Resonance (SPR) has been used to assess an optimized biofunctionalization protocol of gold surfaces with anti-XF, validating their capturing efficacy against *X. fastidiosa*.[3] Among other bio-sensing techniques, SPR holds the advantage of being a label-free detection system, providing real-time monitoring of bio-affinity reactions.[4] So far, a variety of plant pathogen biomarkers were studied by means of SPR, but none of them involves *X. fastidiosa*.[5]

The bio-functionalization of gold transducing interfaces was performed with the polyclonal antibodies for X. fastidiosa, covalently bounded to a self-assembled monolayer of alkylthiols. This configuration guaranteed the assay selectivity, which is assessed by means of a control experiment with the non-binding *Burkholderia phytofirmans* bacterium. The SPR sensogram of the binding *X. fastidiosa* is depicted in Figure 1A, and the comparison with the control experiment response is reported in Figure 1B.

Remarkably, a limit of detection as low as 10^5 CFU/mL was achieved by transducing the direct interaction between the *X. fastidiosa* bacterium and its affinity antibody, which is comparable to the label-needing ELISA gold standard. Moreover, the binding-affinity between polyclonal antibodies and the *X. fastidiosa* bacteria has been also evaluated, obtaining an equilibrium affinity constant of $3.5 \cdot 10^7$ M^{-1}, comparable with those given in the literature for bacteria detection against affinity antibodies.[6] The study is therefore a preliminary development of a reliable cost-effective process to successfully bio-functionalize a gold surface, eventually suitable as gate electrode in wide-field bioelectronic sensors, for ultra-sensitive detection of *X. fastidiosa*.[7]

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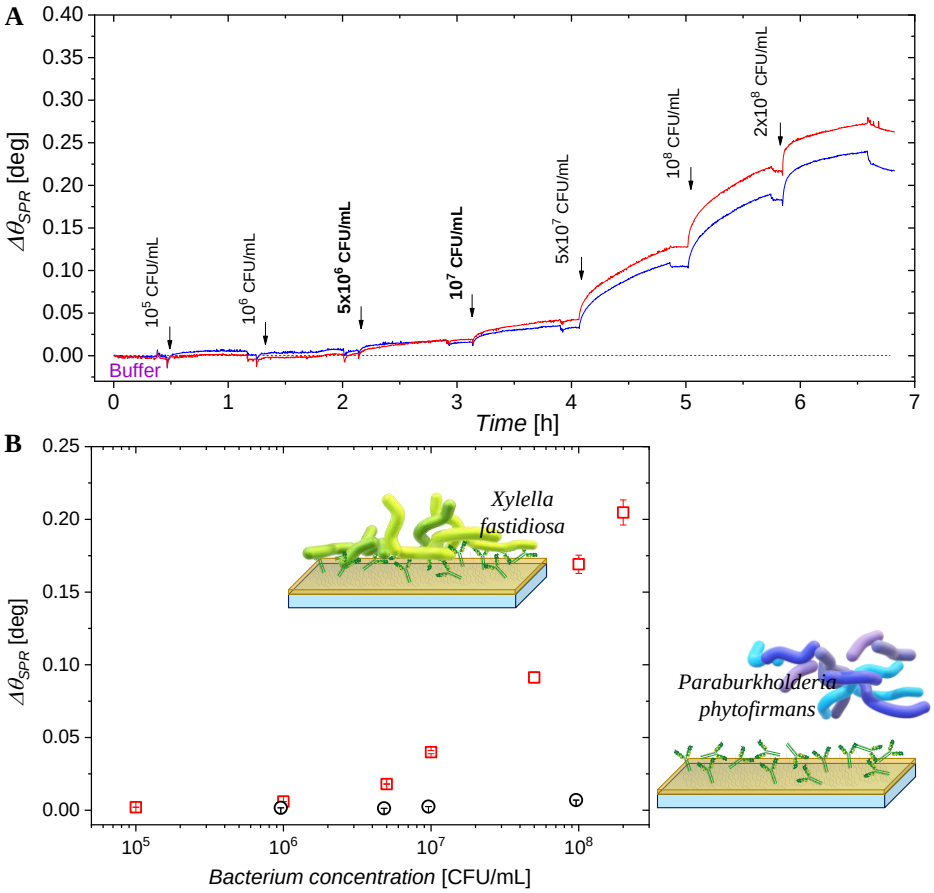

Fig.1 A) Exposure of the anti-XF functionalized surface to *X. fastidiosa* at increasing concentrations; black arrows correspond to sample injections and purple dotted line refers to the buffer level. Red and blue lines refer to two sampling points measured simultaneously. B) Comparison of *X. fastidiosa* (red square) and *Burkholderia phytofirmans* (black circle) SPR responses against the anti-XF functionalized surface. The average signal and standard deviation for four replicates analysis are reported.[3]

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