DOI 10.5162/EUROSENSORSXXXVI/OT6.15

Microfluidic Rolling Circle Amplification Device for Pathogen Detection in Food Products

Rafaela R. Rosa[1], Ana Margarida Fortes[2], Virginia Chu[1], João Pedro Conde[1,2]
[1]Instituto de Engenharia de Sistemas e Computadores – Microsistemas e Nanotecnologias (INESC-MN), Rua Alves Redol, 1000-029, Lisbon, Portugal
[2]BioISI, Faculdade de Ciências de Lisboa, Universidade de Lisboa, Lisbon, Portugal
[3]Department of Bioengineering, Instituto Superior Técnico, Avenida Rovisco Pais 1049-001, Lisbon, Portugal,

joao.conde@tecnico.ulisboa.pt

Summary:
Due to climate change, infections in plants have become increasingly frequent and damaging. For this reason, methods to provide early detection of infection are crucial to allow for timely treatment to prevent the spread of the infection and to safeguard the crop. In this work, we have developed a microfluidic device to amplify DNA through rolling circle amplification using microbeads as a solid platform to immobilize the DNA. We demonstrate the application of this device towards the detection of *Botrytis cinerea*, a pathogen that has a major impact on several crops.

Keywords: microfluidics, microbeads, rolling circle amplification, agrofood, infections

Introduction

Climate changes are having an immense impact in agriculture, which can lead to the loss of crops due to several factors, such as the increase in infections. This can affect the production of several food products, such as grapes, strawberries and tomatoes. Microbiological infections are present in many food products both at the production stage and in the post-harvest processing. Therefore, having a way of detecting these infections early on and employ the appropriate treatments is key, and the development of a device that can detect these pathogens in a fast, low-cost and sensitive way in the field or at the production site is of extreme importance. To achieve these goals, microfluidics is the ideal platform due to its inherent small size, which allows for low reagent volumes, enhanced assay kinetics and low fabrication costs. Additionally, isothermal DNA amplification, particularly padlock-rolling circle amplification (PLP-RCA), is a powerful tool that allows for a very sensitive and specific detection of pathogens and can easily be integrated into a portable microfluidic device to be used at the point of impact. Given that one of the pathogens that greatly impacts several crops is *Botrytis cinerea* it is the targeted pathogen in this work.

Methods and results

In this work we have successfully captured, quantified and amplified our target DNA in a microfluidic system using microbeads as a solid support system. First, a calibration curve for the fluorescence signal emitted by Alexa430 labelled DNA on Q-Sepharose beads was obtained to be used for the quantification of the capture of target DNA (Fig 1.).

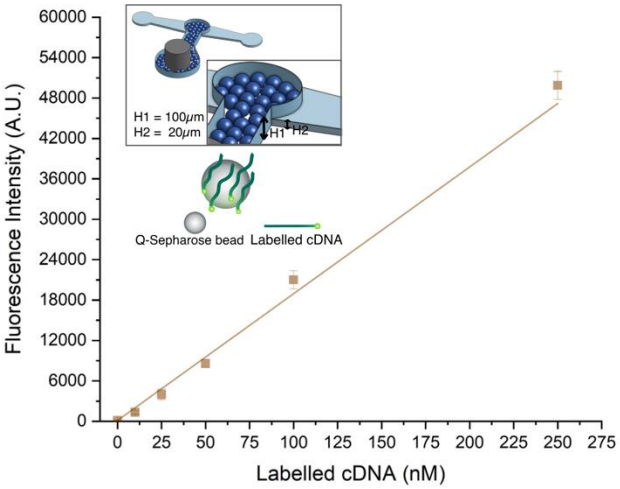

Fig. 1. Calibration curve of the capture of Alexa430 labelled DNA in Q-Sepharose beads.

To evaluate the efficiency and specificity of the PLP, we evaluated two target capture strategies (primer and anchor) with a ssDNA labelled with Alexa430 (Fig 2.). In the primer capture, the biotinylated primer is immobilized on the streptavidin beads followed by hybridization with the back of the PLP, which then circularizes in the presence of the target, which hybridizes with its two ends, if complementary. On the other hand, with the anchor capture, the biotinylated anchor is immobilized on the streptavidin beads followed by

hybridization with a section of the target, then hybridization of the PLP with the target, as in the first strategy. Positive and negative controls for the capture assay were also performed with no biotinylated capture element (primer or anchor) and with a non-complementary labelled DNA, highlighting the specificity of the developed strategies. To assess how much target DNA was captured during the assay, after flowing the labeled ssDNA through the channel with streptavidin beads with previously immobilized anchor or primer and PLP, the solution with the uncaptured target is collected at the outlet and flowed through a second channel with Q-Sepharose beads. From the signal of the labelled cDNA in the Q-Sepharose beads (light blue and green bars in Fig 2.) and using the calibration curve (Fig 1.) and a mass balance strategy, we were then able to calculate how much target DNA was successfully captured, resulting in 87% capture efficiency with the primer capture and 61% with the anchor strategy.

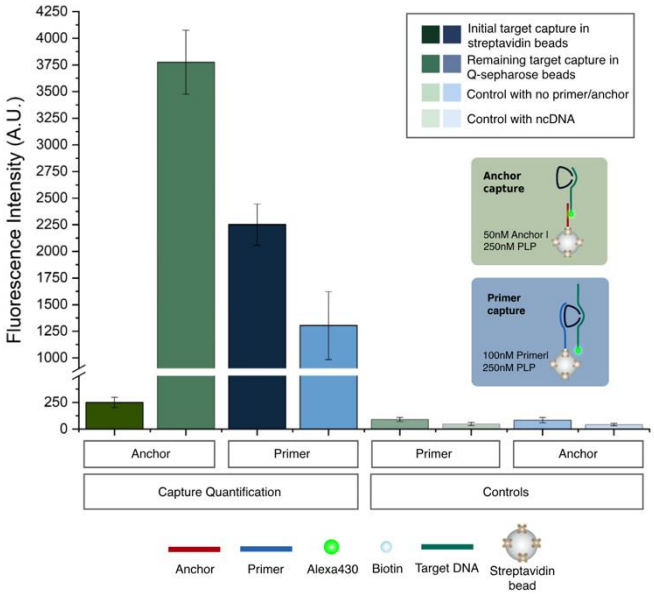

Fig. 2. Target DNA capture and quantification assay for the anchor (in green) and primer capture (in blue) strategy, and respective controls. The capture quantification side of the graphic represents the fluorescence signals obtained in both the streptavidin and Q-Sepharose beads for the capture quantification assay. The controls side of the graphic represents the positive and negative controls.

Lastly, amplification of *Botrytis cinerea* ssDNA (Fig. 3) was also achieved using both target capture strategies (primer and anchor) and RCA, which upon immobilization of the DNA on the microbeads is carried out, by flowing the solutions sequentially. When the PLP hybridizes with the cDNA a gap is left between its two ends which is closed by a DNA ligase. Following the successful ligation, a DNA polymerase with strand displacement activity, carries out the amplification of a long ssDNA strand by going around the PLP multiple rounds. [1] Lastly, the amplification product is detected through the use of oligonucleotides labelled with Alexa430.

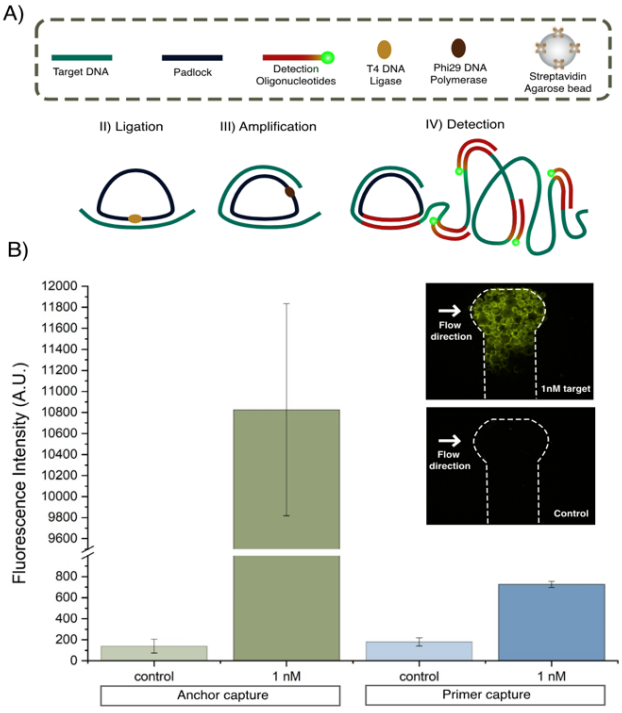

Fig. 3. A) Schematic representation of the RCA assay. B) RCA of Botrytis cinerea ssDNA for both anchor and primer capture strategies, with the controls where no target DNA was flown through the channel with experimental images of the anchor capture positive and control experiments.

Although we were able to successfully detect the target DNA, further optimization will be carried out, to increase the assay sensitivity. A DNA extraction protocol will also be developed and then implemented in the microfluidic device. All of this will allow for pathogen detection upon DNA amplification from real samples, such as grapes.

Acknowledgements

R. Rosa acknowledges FCT for a PhD grant (2022.13021.BD). The authors thank FCT for funding through the project VineSense (PTDC/BAA-DIG/4735/2020) [http://doi.org/10.54499/PTDC/BAA-DIG/4735.INESC MN acknowledges FCT for funding through the Reseach Unit UIDB/0536/2020 and UIDP/0536/2020.

References

[1] R.Soares, N.Madaboosi, M.Nilsson, Rolling Circle Amplification in integrated mycrosystems: An Uncut Gem toward massively multiplexed pathogen diagnostics and genotyping, Accounts of Chemical Research 54, 3979-3990. DOI: 10.1021/acs.accounts.1c00438