Improving Electrochemical Aptasensor Sensitivity for Bacillus cereus Spore Detection in Food Safety Applications

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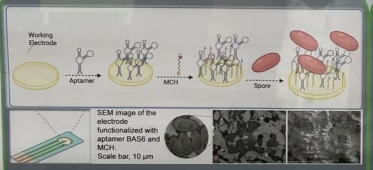
INTRODUCTION

Among food contaminating agents, Bacillus cereus, a Gram-positive spore-forming bacteria, poses serious problems for both consumers and food industry1. It is the primary microbe associated with baby food, third agent responsible for collective foodborne outbreaks in Europe and third leading foodborne pathogen in China1. The most dangerous species of the B. cereus group is Bacillus anthracis, the etiologic agent of anthrax, which causes a highly lethal disease in both humans and animals. Aptamers selected against B. cereus spores can be integrated into diagnostic aptasensors that function without the need for spore germination or lysis. Therefore, we speculate, here, that using a combination of aptamers that recognize B. cereus spores will allow targeting multiple epitopes at their surface, which will provide an enhance diagnostic sensitivity. For this, we tested three different aptamers already characterized for their binding to spores of various B. cereus strains, including B. anthracis, named BAS6R in salade samples.

Schematic concept of the Aptasensor construction

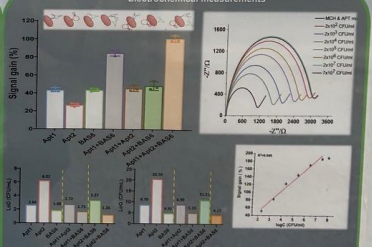


SEM image of the electrode functionalized with aptamer BAS6 and MCH. Scale bar, 10 µm

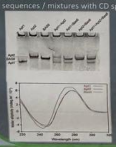
Table 1. Sequences of ssDNA used in this study

Sequence (5' → 3')	Length	Calculated T_m(°C)	Experimental T_m(°C)
Apt1 CATCCGTCACACCTGCTCCGTGCAGACCCATAGGGGGGGGCGTGCGGATGTGCGAGTAGGGTGTTC GCTCCCGTATC	77mer	87.5 °C	86.7 ± 0.5
Apt2 CATCCGTCACACCTGCTCCCCAATGAAGCGAGATGGACGCCTAGCACCCCCCGCGTCCGGTGTTGGC TCCCGTAT	78mer	87.2 °C	41.9 ± 0.6
BAS6 ATCCGTGCACACCTGCTCTGCACGGAGCTCAGTTTGGCTTTGTATCCTAAGAGGATGGTGTTGGCTCCCG TAT	77mer	82.1 °C	31.8 ± 0.6

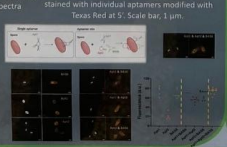
Electrochemical measurements



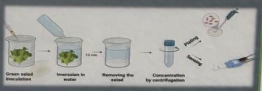
Native gel electrophoresis of different aptamer sequences / mixtures with CD spectra



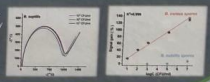
Fluorescence images of B. cereus 551 spores stained with individual aptamers modified with Texas Red at 5'. Scale bar, 1 µm.



Schematic representation of the triple-aptamer based assay for B. cereus spore detection in contaminated ready-to-eat salad samples



Nyquist plots of impedance spectra taken with ferro/ferry cyanide redox couple in mineral water with various concentrations of negative control B. subtilis spores; calibration curves



CONCLUSION

The combination of three aptamers and an impedance-based detection strategy was presented in this study as a novel approach for the diagnostic of B. cereus spores directly from food samples, without the need for bacterial enrichment, culturing, or biomarker purification. The method enabled rapid and ultra-sensitive detection of B. cereus spores at concentrations as low as 1-2 CFU/mL within 15 minutes. This triple-aptamer biosensor has the potential to be used by the food industry and regulatory agencies to monitor food quality, especially considering that different B. cereus strains can cause food spoilage, and infections.

References

1. Authority, E. F. S. (2019). The European Union one health 2018 zoonoses report. EFSA Journal, 17(12), e05926.
2. Cormontagne, D., Rigaud, V., Vidic, J., Rizzotto, F., Bille, E., Bamba, Ramarao, N. (2021). Bacillus cereus induces severe infections in preterm neonates: implication at the hospital and human milk bank level. Toxins, 13(2), 123.