Colorimetric aptasensor for the detection of Bacillus cytotoxicus spores in milk and ready-to-use food

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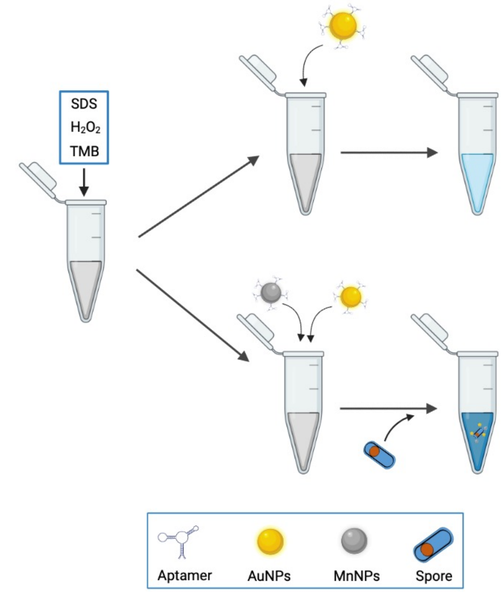
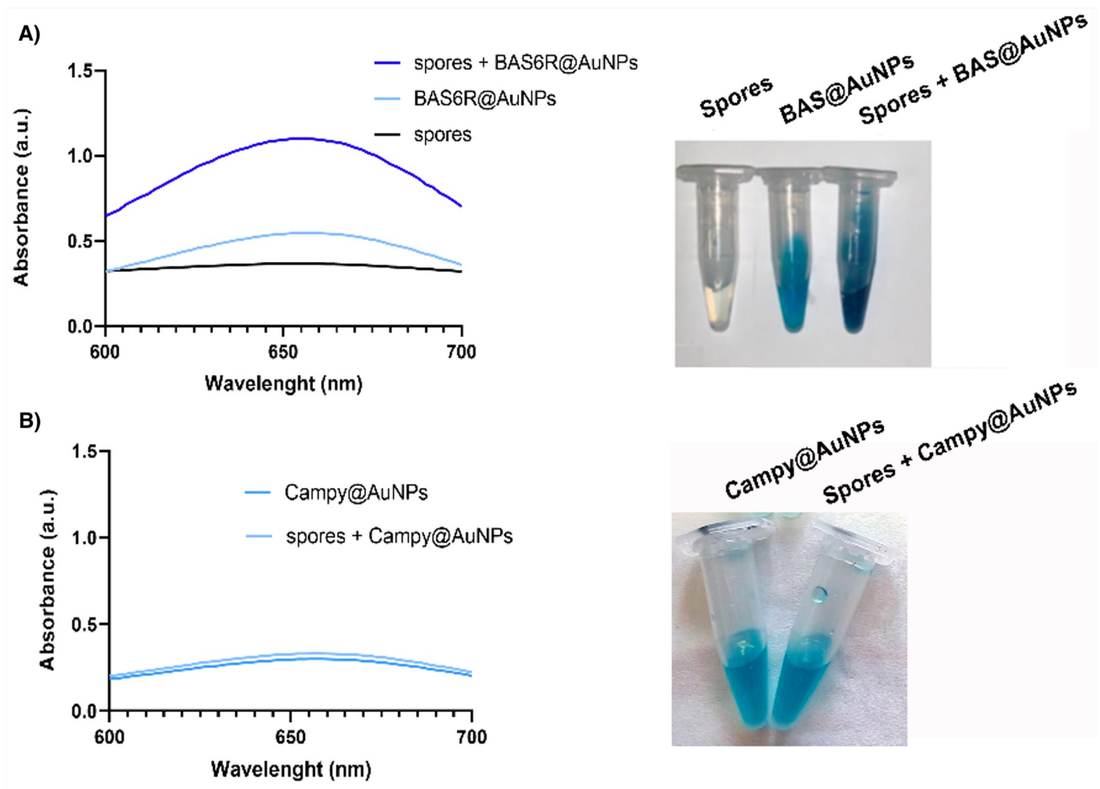
ABSTRACT

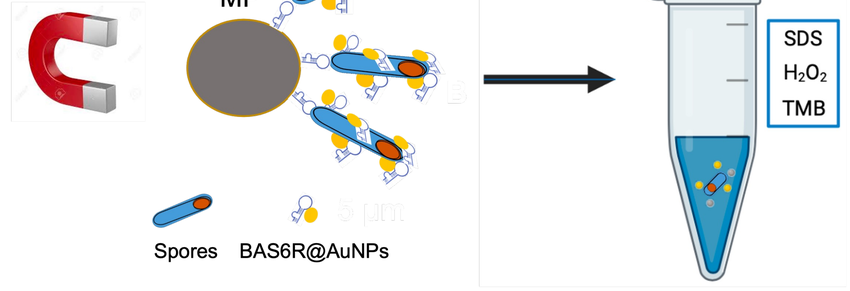
The high incidence of foodborne diseases caused by pathogenic bacteria raises concerns worldwide and imposes considerable public healthcare challenges. This is especially observed with dormant spores of Bacilli, which can often survive treatments used by the food industry to kill growing bacteria. We present a point-of-need colorimetric assay for detection of *Bacillus cytotoxicus* spores in food. The sensing platform consists of a microtube containing gold nanoparticles (AuNPs), and magnetic particles (MPs), both conjugated with specific aptamer BAS6R that recognize *B. cytotoxicus* spores. Upon the addition of the sample, spores were determined as present by the enhanced color change of the solution, due to the oxidation of tetramethylbenidine (TMB) with H_2O_2. BAS6R@AuNPs aptasensor coupled to BAS6R@MPs proved to be highly sensitive, achieving the naked-eye limit of detection as low as 10^2 cfu/mL in water and milk, and 10^4 cfu/mL in mashed potatoes. Moreover, discrimination between spores of *B. cytotoxicus* and *B. subtilis* as well as bacterial vegetative cells was achieved in contaminated food samples, providing a good selectivity. This work provides a promising proof of concept for the development of instrument-free, low-cost and rapid assay for *B. cytotoxicus* spore detection, which is able to compete in sensitivity with conventional costly and time-consuming laboratory analyses.



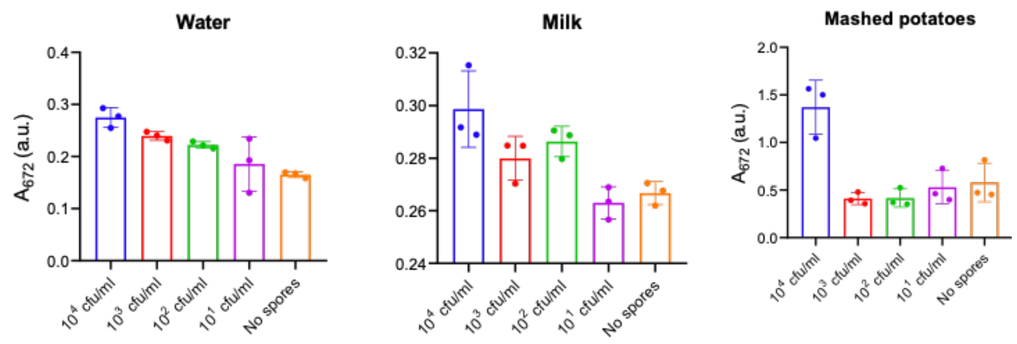
RESULTS



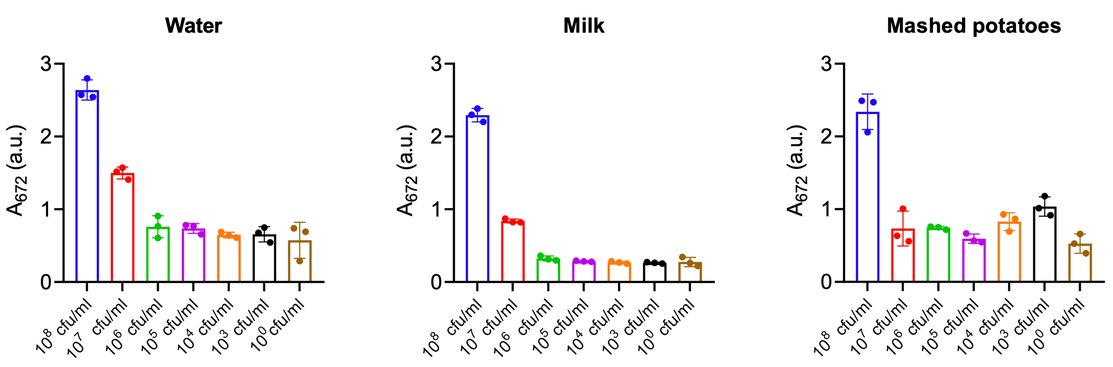
A) UV-Vis absorption spectra of TMB solution containing 10^8 cfu/ml of *B. cytotoxicus* spores, BAS6R@AuNPs (4 x 10^{10} NPs) or their mixture and photographs to illustrate the color change. B) UV-Vis absorption spectra of TMB solution containing Campy@AuNPs (4x10^{10} NPs) and Campy@AuNPs (4 x 10^{10} NPs) with *B. cyctotoxicus* spores (10^8 cfu/ml). Photographs illustrate no color changes



Schematic illustration of *B. cytotoxicus* spores forming into a sandwich between magnetic particles functionalized with BAS-6R (BAS6R@MN) and BAS6R@AuNPs



Detection results for *B. cytotoxicus* spores in three different matrices using BAS6R@AuNPs aptasensor coupled to magnetic concentration. Quantitative evaluation was performed by measuring absorption at 672 nm. Individual data points (n = 3 independent experiments) are shown, error bars represent means ± SD



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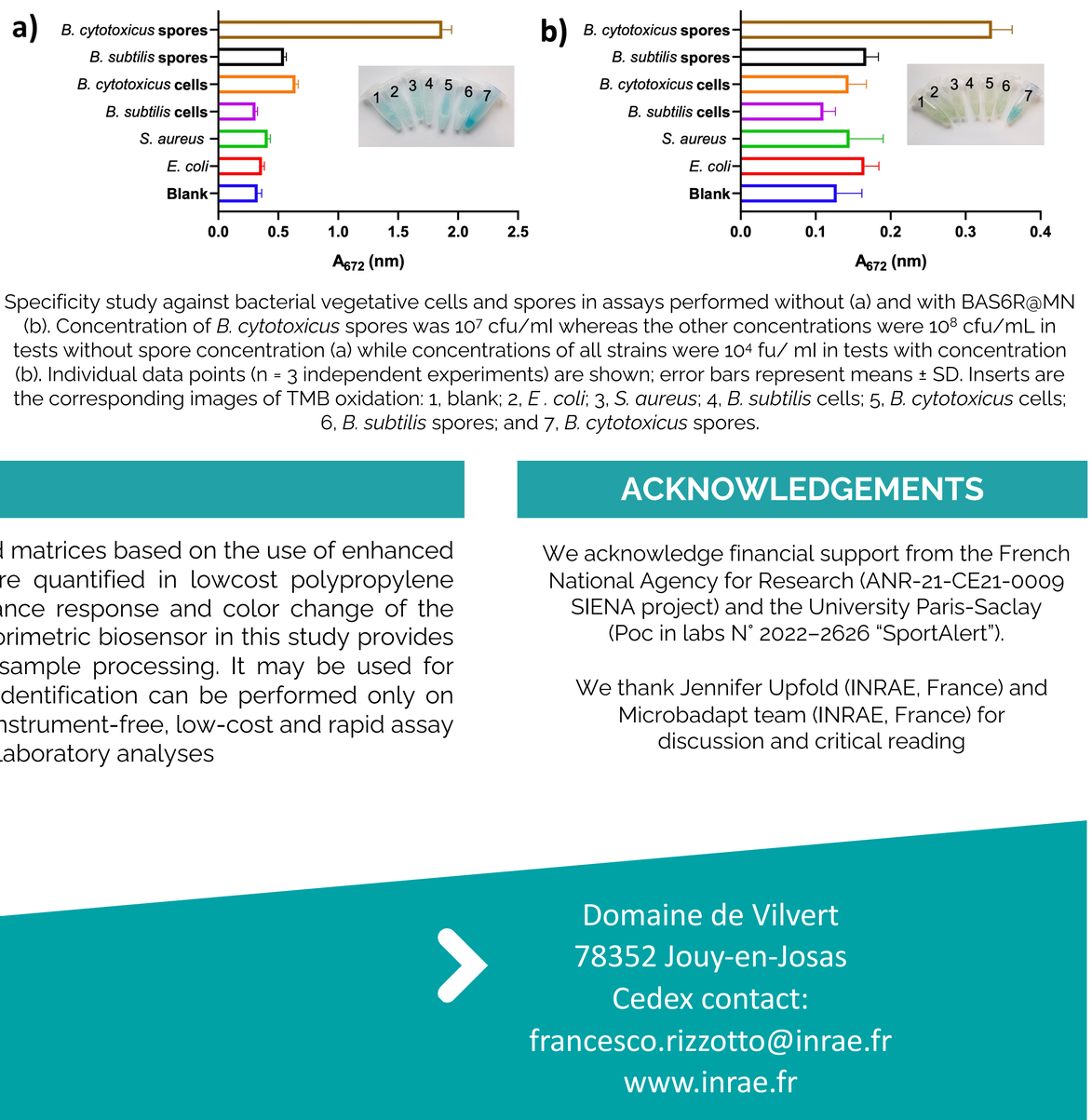

Specificity study against bacterial vegetative cells and spores in assays performed without (a) and with BAS6R@MN (b). Concentration of *B. cytotoxicus* spores was 10^7 cfu/ml whereas the other concentrations were 10^8 cfu/mL in tests without spore concentration (a) while concentrations of all strains were 10^4 fu/ ml in tests with concentration (b). Individual data points (n = 3 independent experiments) are shown; error bars represent means ± SD. Inserts are the corresponding images of TMB oxidation: 1, blank; 2, *E . coli*; 3, *S. aureus*; 4, *B. subtilis* cells; 5, *B. cytotoxicus* cells; 6, *B. subtilis* spores; and 7, *B. cytotoxicus* spores.

CONCLUSION

We constructed an affordable strategy for the simple and rapid detection of *B. cytotoxicus* spores in food matrices based on the use of enhanced peroxidase-like activity of AuNPs. Through this rapid colorimetric test, spores of *B. cytotoxicus* were quantified in lowcost polypropylene microtubes, to a concentration of 10^2 cfu/mL. The detection strategy was demonstrated by absorbance response and color change of the substrate which was catalyzed by AuNPs concentrated on the spores via the specific aptamer. The colorimetric biosensor in this study provides efficient screening method for spores due to lower detection limit, wide linear range and minimal sample processing. It may be used for preliminary monitoring of food products that represent high risk, while confirmation tests and strain identification can be performed only on positive food items. This work provides a promising proof of concept for the development of label-free, instrument-free, low-cost and rapid assay for spore detection which is able to compete in sensitivity with conventional costly and time-consuming laboratory analyses

ACKNOWLEDGEMENTS

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