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Plasmon-assisted fast colorimetric detection of bacterial nucleases in food samples

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ABSTRACT

We have developed a rapid, inexpensive, colorimetric and easy to use method able to detect living bacterial pathogens of zoonotic and foodborne interest. The method is based on detection of bacterial nucleases which cut selectively oligonucleotide probes that are designed in a way to induce aggregation of oligonucleotide stabilised gold-nanoparticles. We present the standardization of our method to detect nucleases secreted by Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Salmonella* spp.) pathogens, not only in five different matrixes of food samples experimentally contaminated but also in naturally contaminated foodstuffs. Our method has shown sensitivity/specificity, detecting nucleases in less than two hours in supernatants from bacterial cell culture (1 CFU/mL) incubated for 15 h. The nucleases are detected by naked-eye inspection, and using minimal laboratory equipment. From a broader perspective, besides applications in foodstuff safety, we envision a potential use of our method to detect other bacterial and viral pathogens in the environment, and in veterinary and human health.

1. Introduction

Foodborne diseases create serious concerns in public health worldwide [1,2], particularly, considering the acceleration at which foodstuffs move through the production and distribution chain all around the world. The most relevant foodborne pathogen bacteria are *Salmonella*, *Listeria*, *Campylobacter*, as well as certain *Staphylococcus aureus* (*S. aureus*) and *E. coli* [1–4]. To control these bacteria is a priority not only for world health authorities but also for the global food industry, whose prime interest is to ensure the food safety of consumers. To achieve this, preventive approaches are currently applied, such as implementation of good hygiene practices and application of procedures based on hazard analysis and critical control point (HACCP) principles. In fact, the European Commission Regulation 2073/2005 lays down microbiological criteria for certain microorganisms and implementation rules to be complied by food business operators. Similarly, the Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) recommends that microbiological criteria should be relevant and effective in relation to consumer's health protection [5].

The methods presently available for detection of foodstuff pathogens involve well-standardised microbiological culture techniques that allow the isolation of the pathogen. These methods, however, suffer from low sensitivity and high cost; they are also time consuming, labour intensive and require well-trained personnel plus the readiness of biosafety facilities. For example, detection of *Salmonella* i.e. one of the main foodborne pathogens worldwide, causing yearly 100,000 human cases in Europe [6,7] and 1.2 million estimated cases in the USA [8] is regulated by the standard ISO 6579:2002 technique [9] that allows the irrefutable

Abbreviations: *S.* Typhimurium and *S.* Enteritidis, *Salmonella enterica* subspecies *enterica* serovar Typhimurium and serovar Enteritidis respectively; *S. aureus*, *Staphylococcus aureus*; CFU, Colony forming units; LOD, Limit of detection.

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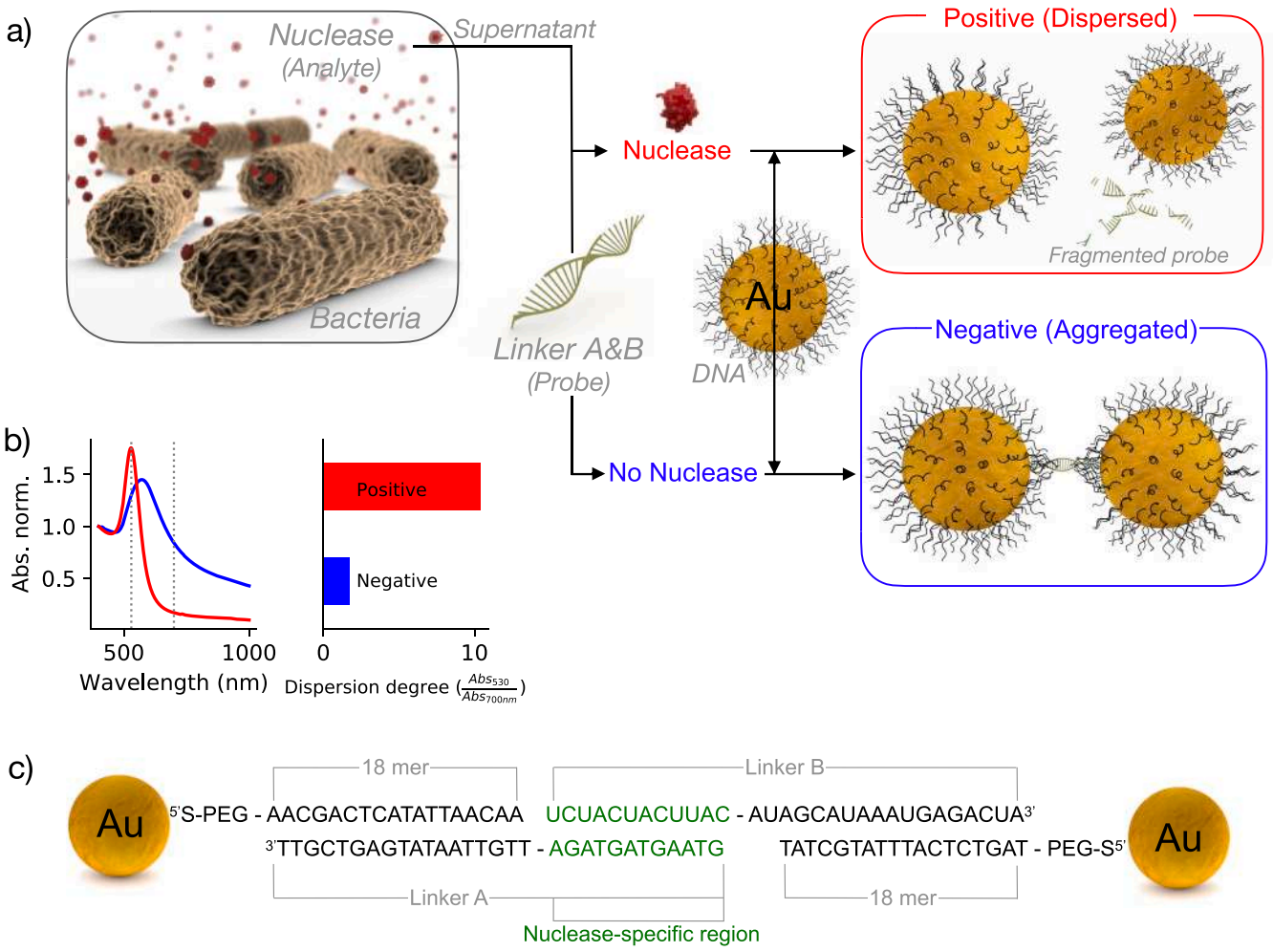

Fig. 1. Schematic representation of the working principle of the colorimetric assay. (a) Nucleases produced by the pathogen recognise and degrade specific regions of the designed oligonucleotide probe (Linkers A&B), thus, preventing aggregation of Au@DNA. In absence of nucleases, oligonucleotide probes remain intact, aggregating Au@DNA; (b) Assay readout. left: absorbance values at different wavelength of the UV-Vis-NIR spectra of positive (red) and negative (blue) results; right: Dispersion degree (D = Abs_{530}/Abs_{700} – dotted lines in left plot) obtained with positive (in red) and negative (in blue) samples, as a qualitative measure of sensor performance; (c) Architecture of components bind to the Au@DNA stabilised with 18-mer capture probes that are complementary to the exemplified Linker A-DNA and Linker B-2′OMe. Both linkers hybridise via the nuclease-specific region (marked in green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

isolation of the pathogen. It has low sensitivity [10] and requires handling enriched cultures of the pathogen in six sequential steps, using different culture media and biochemical reagents, by a well-trained microbiologist and, at least, one week to isolate suspected colonies, as well as additional period for confirmation and typing in a reference laboratory. Such lengthy protocols hamper early detection of foodstuff contaminant pathogens, making urgent the availability of a rapid, cost-effective, sensitive, specific, and easy-to-use system able to detect live bacteria early on in the food chain, especially in those products with a short shelf-life.

To meet the need for a new detection system, several approaches have been proposed that are based either on DNA amplification or on antigens immune-detection [11,12]. However, these bacterial molecules may remain in food even after sterilization, thus, leading to identify the foodstuff as contaminated when the pathogen is no longer alive. Recently, nucleases secreted by living microorganisms have become an emerging biomarker able to digest specific oligonucleotide fragments (oligoprobes) through cleaving phosphodiester bonds between nucleic acids [13]. Fluorescent assays have been developed for targeting microorganisms, using chemically modified oligoprobes that undergo selective fragmentation in the presence of nucleases secreted by live bacteria [13–17]. These fluorescent-based methods have some inconveniences, such as requiring: (i) oligoprobes carrying a fluorophore and quencher that make the detection cost-intensive; (ii) the use of special fluorometric equipment; (iii) be carried out by qualified training personnel; (iv) high cost. However, oligoprobes can be readily modified, offering an excellent potential technology for real-time analysis to detect live pathogens.

Gold nanoparticles exhibit unique optical properties arising from localised surface plasmon resonance in the visible spectral range, rendering them a convenient colorimetric signal transducer that can be used to detect pathogens present in food, water, cleanrooms surfaces or environment [18–27]. These colorimetric systems have been used to detect DNA or bacterial antigenic determinants. To the best of our knowledge, however, the detection of bacterial nucleases by the combination of DNA technology and plasmonic nanoparticles remains without experimental demonstration [28–33].

In this paper, we report a new label-free colorimetric method for the detection of nucleases produced by live bacteria, using aggregating gold nanoparticles as an optical signal transducer. Our assay comprises an enzyme-susceptible oligonucleotide duplex probes that is degraded by bacterial nucleases, preventing the aggregation of DNA-stabilised gold

Table 1
Oligonucleotide sequences analysed in the present study.

Name	Oligonucleotide Sequence (5′ to 3′)
Oligonucleotides conjugated on nanoparticles:	
DNA A*	SH-Spacer18-AAC-GAC-TCA-TAT-TAA-CAA
DNA B*	SH-Spacer18-TAG-TCT-CAT-TTA-TGC-TAT
Oligonucleotides for NP-based nuclease assay:	
Linker A-DNA[a]	GTA-AGT-AGT-AGA-TTG-TTA-ATA-TGA-GTC-GTT
Linker A-RNA	GUA-AGU-AGU-AGA-UUG-UUA-AUA-UGA-GUC-GUU
Linker A-2′OMe	mGmUmA-mAmGmU-mAmGmU-mAmGmA-mUmUmG-mUmUmA-mAmUmA-mUmGmA-mGmUmC-mGmUmU
Linker B-DNA	TCT-ACT-ACT-TAC-ATA-GCA-TAA-ATG-AGA-CTA
Linker B-RNA	UCU-ACU-ACU-UAC-AUA-GCA-UAA-AUG-AGA-CUA
Linker B-2′OMe[a]	mUmCmU-mAmCmU-mAmCmU-mUmAmC-mAmUmA-mGmCmA-mUmAmA-mAmUmG-mAmGmA-mCmUmA
Linker C-DNA	GCA-AGC-AGC-AGA-TTG-TTA-ATA-TGA-GTC-GTT
Linker C-RNA[b]	GCA-AGC-AGC-AGA-UUG-UUA-AUA-UGA-GUC-GUU
Linker D-2′OMe[b]	mUmCmU-mGmCmU-mGmCmU-mUmGmC-mAmUmA-mGmCmA-mUmAmA-mAmUmG-mAmGmA-mCmUmA
Linker E-DNA	GAA-AGC-AGG-AGA-TTG-TTA-ATA-TGA-GTC-GTT
Linker E-RNA	GAA-AGC-AGG-AGA-UUG-UUA-AUA-UGA-GUC-GUU
Linker F-2′OMe	mUmCmU-mCmCmU-mGmCmU-mUmUmC-mAmUmA-mGmCmA-mUmAmA-mAmUmG-mAmGmA-mCmUmA
Oligonucleotides for fluorescence-based nuclease assay:	
FAM	6-FAM-dGdGdG-rUrUrU-3′DABCYL

* Sequences modified at the 5′-end with a thiol group followed by a polyethylene-glycol spacer followed by the nucleotide sequence (SH-Spacer18-nucleotide sequence).
[a] Linkers eventually selected for *S. aureus* detection;
[b] Linkers eventually selected for *Salmonella* spp. detection.

nanoparticles and conservation of initial red colour of the colloidal suspension (positive readout) (Fig. 1). In the absence of bacterial nucleases, nanoparticles aggregate, causing colour change from red to blue (negative readout). Key benefits are: it is rapid; cost-effective and easy-to-use. By rational design of the oligoprobe, our method is capable of detecting nucleases secreted either by a wide diversity of *Salmonella* species, including *Salmonella enterica* subspecies *enterica* serovar Typhimurium and serovar Enteritidis (*S*. Typhimurium and *S*. Enteritidis, respectively, ahead) or by *S. aureus*. Both Gram-negative and Gram-negative types of pathogens were detected, not only under experimental conditions, but also in naturally contaminated food samples.

2. Materials and methods

2.1. Chemicals

Hydrogen tetrachloroaurate trihydrate ($HAuCl_4.3H_2O$) was purchased from Alfa Aesar (Thermo Fisher Scientific, Spain). Sodium dodecyl sulphate (SDS) 98%, sodium chloride (NaCl) 99.5%, and phosphate buffer (PB) 1 M, pH 7.4, were purchased from Sigma-Aldrich (Spain). Phosphate buffered saline (PBS) 0.01 M, pH 7.4, containing 0.138 M NaCl and 2.7 mM KCl (Sigma-Aldrich) was used to mimic physiological conditions, either plain (PBS -/-) or supplemented with $MgCl_2$ and $CaCl_2$ (PBS +/+) (Gibco, Life Technologies, Spain). Luria Bertani (LB), Tryptic Soy Broth (TSB) and Buffered Peptone Water (BPW) culture media were purchased in Difco (Pronadisa, Spain). DNA sequences and thiolated-oligonucleotides were purchased from Biomers (Germany).

2.2. Instrumentation

UV-Vis-NIR spectra were measured at room temperature on a Jasco and Cary 3500 UV-Vis-NIR spectrophotometers, using UV Micro cuvettes with 1 cm optical path length. Transmission electron microscopy (TEM) analysis was performed by JEOL JEM-1400 PLUS, operating at 120 kV. Dynamic light scattering (DLS) measurements were carried out in a Malvern NanoSizer. A Veriti Thermal Cycler 60-well Applied Biosystems was used for the incubation of the samples. Emission spectra were obtained by a Synergy Neo2 Multimode Reader Biotek.

2.3. Synthesis of gold nanoparticles

Following a seeded growth method [34], the synthesis of the Au seeds was performed with trisodium citrate solution (150 mL, 2.2 mM) heated for 15 min under vigorous stirring until boiling, followed by the injection of $HAuCl_4$ (1 mL, 25 mM). In such conditions, the colour of the solution changed from yellow to bluish grey and then to light pink in 10 min. Thereafter, the seeded growth process comprised cyclic addition of metal precursor and nanoparticles extraction, by cooling to 90 ºC and then adding $HAuCl_4$ (1 mL, 25 mM) in two steps 30 min apart. After additional 30 min, 55 mL of the growth solution were removed and the remaining 98 mL were mixed with water (53 mL) and sodium citrate (2 mL, 60 mM). This process was repeated 2 times to obtain nanoparticles of ≈ 26 nm gold core diameter.

18-mer DNA functionalisation of gold nanoparticles by Hurst et al. [35]. An excess of thiolated oligonucleotides (DNA A or DNA B, Table 1, 0.1 mM) was added to the gold nanoparticles colloid (5.55 mL) containing SDS 0.1% and PB 0.01 M, followed by the incubation at room temperature for 20 min. To improve the oligonucleotide binding, a salt aging process was carried out by sequential addition of 25 μL, 25 μL, 75 μL, 125 μL and 250 μL of a solution containing NaCl 2 M, SDS 0.01% and PB 0.01 M, up to reach a final concentration of NaCl 0.2 M. Each salt aging step was sonicated (10 s) and incubated (20 min), and the last one was incubated for 12 h, at 25 ºC. To remove the excess of oligonucleotides, the solutions were centrifuged (7000 rpm, 30 min) and re-dispersed in 3 mL of SDS 0.01%. This step was repeated for three times. The final concentration of Au@DNA-A and Au@DNA-B nanoparticles was 0.8 mM in terms of Au atoms for all samples.

2.4. Characterization of gold nanoparticles

As-prepared and functionalised Au@DNA nanoparticles were characterised by TEM and DLS. For TEM, 50 μL of nanoparticles were drop casted onto copper grid placed on filter paper and left for drying. The average metal core diameter was determined by measuring > 100 nanoparticles on TEM micrographs. The hydrodynamic diameter of the Au@DNA nanoparticles was determined by 6 measurements of DLS with 5 runs of 5 s each. The study was carried out at 25 ºC with 120 s of sample equilibration.

2.5. Standardisation of UV-Vis-NIR spectral readouts

We compared the naked eye colour of the Au@DNA positive and negative results with respect to the UV-Vis-NIR spectral readouts, concluding that dispersion degree values of $D \geq 5$ allowed for detection of bacterial nucleases. While the lower limit fell in the range of 1.8–2.1 D values, the upper limit was around D values of 10–15, being the latter sensitive to the monodispersity of the Au@DNA nanoparticles, indicating that the better the quality of nanoparticles, the higher the D values in presence of nucleases.

2.6. Preparation of bacterial supernatants for nuclease assay

Bacterial supernatants were obtained by LB culturing (37 ºC, overnight, under shaking at 200 rpm) and centrifugation (6000 g, 20 min, 4 ºC). For biosecurity purposes, each supernatant was removed, filtered in a 0.2 mm Millipore® (Sarsted) and kept sterile at 4 ºC until its use. To confirm the absence of bacteria, 100 μL of each filtrate were plated on TSA (37 ºC, 48 h).

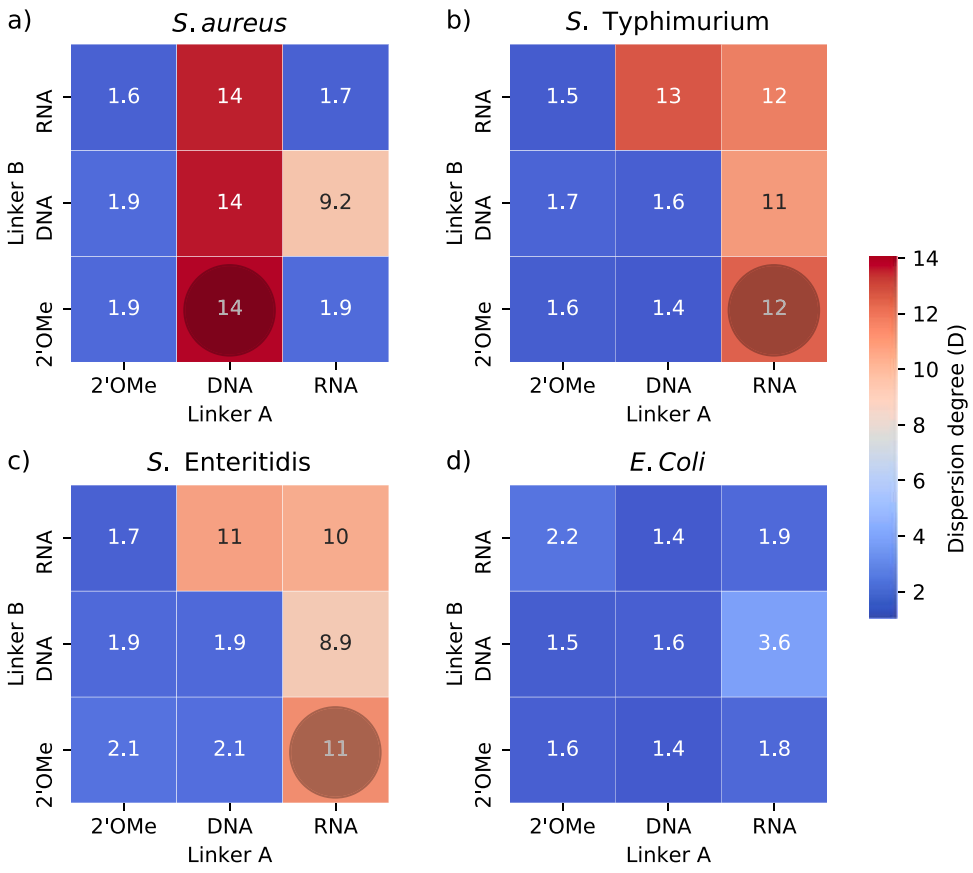

Fig. 2. Coarse screening of different combination of linkers A and B. Bacterial supernatants from (a) *Staphylococcus aureus*, (b) *Salmonella* Typhimurium, (c) *Salmonella* Enteritidis, and (d) *Escherichia coli* (strains Sa1, STM1, SE1 and NS1, respectively, of Tables S1 and S2) were mixed with different duplex combinations of 2′OMe, DNA and RNA linkers A and B. For each duplex are shown the values of dispersion degree and color-coded readout: positive in red for D≥ 5). Shaded circles indicate the best-performing linkers combination selected for further experiments with each pathogen. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.7. Bacterial growth curves

S. aureus and *Salmonella* spp. stocks were cultured (37 °C, 24 h) in LA plates and, then, harvested and adjusted by spectrophotometry (OD_{600}nm = 0.160) to 1 × 10^8 CFU/mL in BPW. Eight serial ten-fold dilutions from this suspension were prepared and those corresponding to 1, 10, 100 or 1000 CFU/mL were used for different experiments. The bacterial growth curves of each bacterial suspension were determined at 0, 2, 4, 6, 8, 10, 12, 24 and 48 h after incubation at 37 °C, by the number of CFU/mL (i.e. serial dilutions in BPW, triplicate plating and incubation of plates 24 h, 37 °C) at each selected time point. The individual number of CFU/mL was logarithmically transformed and the results were expressed as the $\log_{10}$ CFU/mL.

2.8. Colorimetric detection of nucleases in bacterial supernatants

An aliquot of bacterial supernatant (35 μL) was combined with both linkers (3 μL, 1 μM) in sterile PCR tubes (0.5 mL) to give total volume of 41 μL. The mixture was placed in thermal cycler at 37 °C for 60 min. After completing the exposition step, BPC water (32 μL), both types of Au@DNA (12.5 μL of each batch) and NaCl (2 μL, 5 M) were added sequentially. The mixture was left undisturbed at 25 °C for 30 min followed by naked-eye inspection and characterisation by UV–Vis–NIR.

2.9. Salt titration experiment in nuclease-free conditions

An aliquot of each linker (1 μL, 10 μM) was diluted 1:10 with PBS (+/+) up to final volume of 10 μL, which were mixed with 225 μL milli-Q water plus 50 μL of batch DNA-A and 50 μL of DNA-B (Table 1) and NaCl 5 M, in order to reach a final salt concentration ranging from 0 to 0.42 M, in a 0.07 M interval. This solution was incubated for 30 min and then characterised by UV–Vis–NIR. The optimal salt concentration was determined as that onset a dispersion degree value ≈ 2.

2.10. Determination of the optimal exposition time between nucleases and linkers

Supernatants of 10–10^3 CFU/mL *S.* Typhimurium ATCC 14028 were obtained after different incubation intervals from 0 to 48 h and exposed to pair linkers for 30 and 60 min of exposition time. Each mixture was then combined with both batches of Au@DNA nanoparticles (DNA-A and DNA-B, see Table 1), at 0.21 M NaCl. After 30 min of Au@DNA aggregation, the readouts of dispersion degree were measured and graphically represented by colours indicating the gradient of positivity from blue to red (threshold D=5).

2.11. Estimation of the Limit of Detection (LOD) of nucleases by the colorimetric method

Aliquots of commercial S7 micrococcal nuclease (Sigma-Aldrich) and of concentrations ranging from 0 to 75 μUI/μL were incubated with Linker A-DNA and Linker B-2′OMe (Table 1) for 20 min. Thereafter, 50 μL of each Au@DNA nanoparticles were diluted in 225 μL milli-Q water and 25 μL of NaCl 5 M. The LOD was determined by the regression line and the formula LOD = 3 SD /a, where SD was the standard deviation and *a* was the mean value of the blank PBS sample.

2.12. Growth curves of S. aureus and Salmonella spp. in food samples

Samples of cheese, chicken, lettuce, omelette and potato salad were experimentally inoculated with 10^3 CFU/mL of *S. aureus* or *S.* Typhimurium in BPW homogenates (1:10). Contaminated food samples were incubated at 37 °C and viable bacteria (UFC/mL) were collected at 0, 4, 8, 14, 18 and 24 h post-incubation. Aliquots of each suspension were

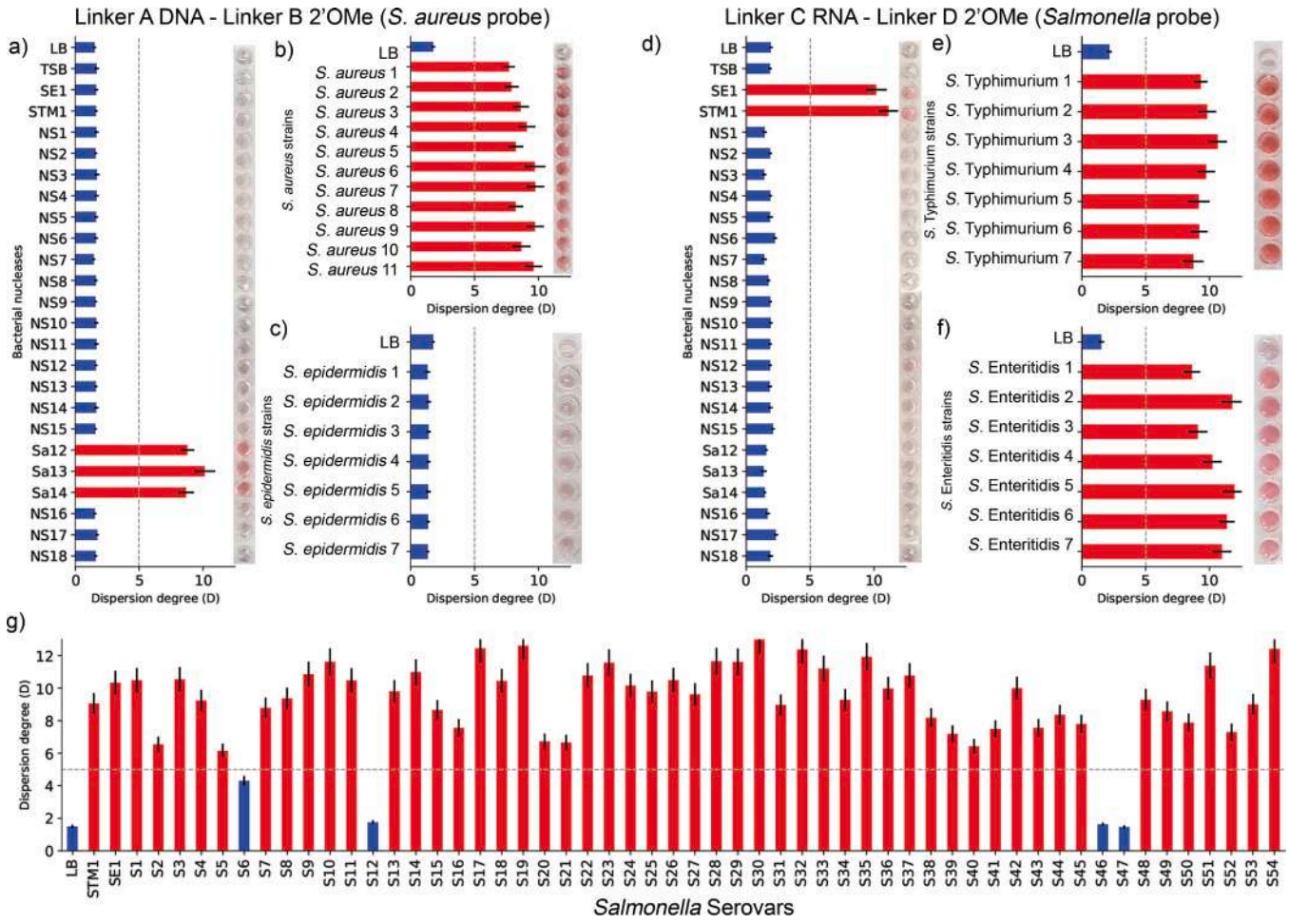

Fig. 3. Selective discrimination of *S. aureus* and *Salmonella* spp. from other bacteria nucleases by using our assay. The duplex linker comprising Linker A-DNA and Linker B-2′OMe allowed the specific detection of (a,b) *S. aureus* strains, discriminating it from (c) S. epidermidis and (a) Non-Staphylococcal bacteria (SE1-SE7 and NS1-NS18 of Table S1, respectively). The duplex linker comprising Linker C-RNA and Linker D-2′OMe allowed the specific detection of (e) *S*. Typhimurium, (f) *S*. Enteritidis, as well as (g) other 50 *Salmonella* enterica subspecies and serovars (Table S2), discriminating them from (d) Non-*Salmonella* bacteria (Sa12-Sa14 and NS1-NS18 of Table S1). Each bar represents the dispersion degree value (threshold at D = 5) and is accompanied by a naked eye digital image of a plastic-well containing the assay mixture.

filtered and kept at 4 °C until its use to determine the presence of nucleases (see above).

2.13. Fluorescence-based nuclease assay

Fluorescent probe comprised 5′ carboxy fluorescein (6-FAM) and a 3′ 4-(dimethylaminoazo)benzene-4-carboxylic acid (DABCYL) that quenched FAM fluorescence. The fluorescein and DABCYL units were separated by six nucleotides susceptible to nucleases, and contained three deoxyribonucleotides and three ribonucleotides (Table 1). To detect the presence of nucleases bacterial supernatant (90 μL) and fluorescence probe (10 μL, final concentration 1 μM). The emission spectra were recorded for 100 min in 60 s interval. In all measurements the excitation and emission wavelengths were fixed to 490, 518 nm, respectively, and applying gain of 90.

3. Results and discussion

3.1. Optimal conditions standardisation

The working principle of our diagnostic method is based on the aggregation of two types of DNA-stabilised gold nanoparticles coated by 18-mer DNA probes (DNA-A and DNA-B, Table 1) through sandwich-type hybridisation with DNA or RNA duplex linkers (Fig. 1c). In a typical detection event, first the duplex linkers are exposed to the supernatant of a microbial culture (30 min of exposition time, at 37 °C), followed by the addition of the two types of Au@DNA nanoparticles (Fig. 1); if the supernatant contains the specific nuclease, the probe undergoes fragmentation, impeding the Au@DNA aggregation (positive reaction, Fig. 1b). In absence of the nuclease, the intact probe built by the duplex linkers joints Au@DNA leading to aggregation over 20 min of readout time (negative outcome). Importantly, a naked eye inspection by an untrained person suffices for the valid readout of our assay. To quantify the performance of the assay we analysed UV-Vis-NIR spectra of the solutions 20–30 min after the addition of Au@DNA nanoparticles, by expressing a unitless value of dispersion degree, $D = Abs_{530}/Abs_{700}$ (Fig. 1b). A threshold of $D \geq 5$ was assigned for a positive outcome.

As an optical signal transducer, we used two batches of gold nanoparticles (26.6 ± 3.3 nm) functionalized with 18-mer DNA capture probes (Table 1). The hydrodynamic diameters of Au@DNA-A and Au@DNA-B was 52.2 ± 0.6 nm and 50.9 ± 0.6 nm, respectively (Fig. S1).

The duplex linkers (probe) contained a total of 30 bases of which 12 were complementary one to each other, and the remaining 18 bases were complementary to capture probes stabilizing Au@DNA. As a coarse screening of probe susceptibility to nucleases, we used DNA, RNA and 2′-O-Methyl (2′OMe) modified sequences (Table 1).

We evaluated all possible combinations of the designed Linkers A

and B described in Table 1, against three different bacterial culture supernatants (i.e., *S. aureus*, *S.* Typhimurium, *S.* Enteritidis) giving 9 different combinations for each supernatant. Sterile LB and *E. coli* broth cultures were used as blank and negative controls, respectively. Fig. 2 displays the values of dispersion degrees (D values), highlighting from negative (blue) to positive (red) readouts. Overall, the presence of DNA oligonucleotides showed clear preferences to detect *S. aureus* nucleases, while linkers comprising RNA oligonucleotides showed preference to detect *S.* Typhimurium and *S.* Enteritidis nucleases. These results are in agreement with previous works in which DNA probes were degraded by a *S. aureus* micrococcal nuclease [14] while RNA probes were degraded by RNases in *Salmonella* supernatants [13].

To investigate the effect of the linker sequence on assay performance we altered the sequence of the linkers (shaded circles in Fig. 2), maintaining the type of nucleic acid: DNA, RNA or 2′OMe. The three first thymines of Linker A-DNA were substituted by three cytosines, leading to Linker C sequence. Two cytosines in Linker C were substituted by an adenine and a guanine, leading to Linker E (see Table 1 for full sequence composition). These modifications in Linkers C and E sequences were associated with changes in the complementary sequences, leading to Linkers D and F, respectively. We observed that altered sequences maintained similar selectivity toward nucleases from both *S. aureus* (Fig. S2a) and *S.* Enteritidis or *S.* Typhimurium (Fig. S2b), indicating that the bacterial nucleases activity was susceptible to the oligonucleotide modification rather than to their sequence. Accordingly, for further experiments, we selected the following pairwise probe composition: duplex linkers A-DNA and B-2′OMe for *S. aureus*; and duplex linkers C-RNA and Linker D-2′OMe for *Salmonella* spp. (Table 1).

Since DNA-driven aggregation of nanoparticles is salt-dependent, the sensor requires the use of an optimal NaCl concentration. At low NaCl concentrations, the duplex linker is unable to aggregate nanoparticles, leading to a positive readout regardless of the presence of nucleases in the solution; and, oppositely, non-specific aggregation of nanoparticles can occur at too high NaCl concentration (>0.5 M) leading to a false negative outcome. We performed a salt titration experiment in nuclease-free conditions, using both types of Au@DNA and the duplex linkers specific to detect *S. aureus* or *Salmonella* described in Table 1. As result, we determined that ranges from 0.2 to 0.3 M of NaCl were optimal (Fig. S3). Note that *Salmonella* probes containing RNA and 2′OMe Linkers C & D required lower amount of salt than *S. aureus* probes, suggesting that RNA sequence was more sensitive to charge screening as compared to DNA.

To determine the optimal exposition time (i.e. minimum time for enzyme-driven linkers fragmentation) we exposed Linker C and Linker D for 30 and 60 min with supernatants obtained after incubation intervals from 0 to 48 h for different *S.* Typhimurium concentrations (i.e. 1–10, 10–100 and 100–1000 CFU/mL). Regardless of the exposition times (30 or 60 min), the assay tested positive for all supernatants obtained at the incubation times above 18 h (Fig. S4). At 60 min-exposition, the method also allowed the detection of nucleases in supernatants obtained after 12–14 h of incubation from the highest bacterial concentration (10^3 CFU/mL). For the sake of practicality and to shorten the operation time of the assay we fixed the exposition time to 30 min, considering also that 24 h of bacterial incubation to obtain the supernatants are more practical than of 12–18 h periods for conventional labour journeys.

3.2. Specificity of the method

Next, we tested the specificity of the colloidal assay developed for *S. aureus* and *Salmonella* spp. by using supernatants from 25 non-*S. aureus* and non-*Salmonella* strains of 14 different bacterial genera (Table S1), as frequent food contaminants. The probe comprising Linker A-DNA and Linker B-2′OMe gave 100% specificity, leading to a positive readout only for *S. aureus* (Fig. 3a). The same probe showed specificity toward 11 other strains of *S. aureus* (Fig. 3b) and negative results for 7 strains of *S. epidermidis* (Fig. 3c). Similarly, strains STM1 and SE1 of *S.* Typhimurium and *S.* Enteritidis, respectively, were specifically detected by this method (Fig. 3d) as well as other 7 different *S.* Typhimurium and *S.* Enteritidis strains (Fig. 3e, f), using the probe comprising Linker C-RNA and Linker D-2′OMe. The same linker combination was used for the detection of 4 subspecies and 50 different serovars of *Salmonella* (Table S2). As shown in Fig. 3g, only 4 serovars of minor epidemiological relevance, i.e. *S.* Coeln, *S.* Wien, *S.* Amsterdam and 6,7:-:1,5 (strains S6, S12, S46 and S47, respectively; Table S2) gave negative results.

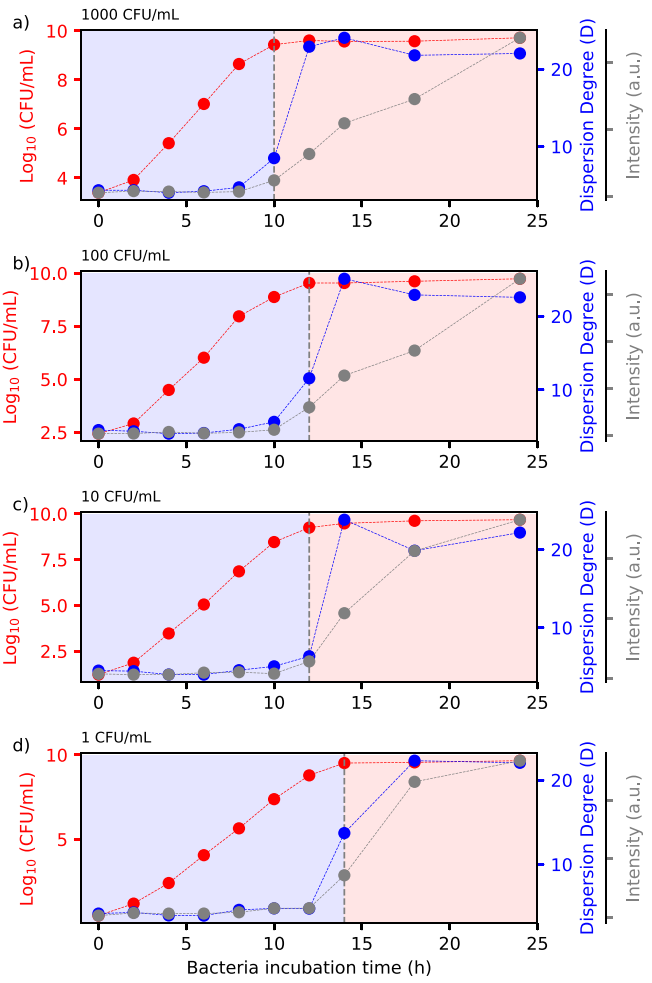

Fig. 4. *S.* Typhimurium bacterial growth curves in BPW and detection of nucleases through plasmonic and fluorescence assays. Red curves: number of viable bacteria ($\log_{10}$ CFU/mL), obtained by serial dilutions and plating of suspensions of *S.* Typhimurium ATCC 14028 ranging from 10^3 to 10^0 CFU/mL (a-d) in BPW (37 °C) at the selected intervals of the incubation. Blue and grey curves: Detection of nucleases in the supernatants at a given CFU/mL and incubation time using plasmonic (blue) and fluorescence assay (grey). Dotted vertical lines represent the threshold of positivity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Noteworthy, we found that a probe containing 2′OMe modification in both linkers gave negative outcome for all the samples tested (Fig. S5), suggesting such linker combination as a negative control for practical purposes. Overall, these results show that our method allows a fast colorimetric detection of bacterial nucleases in less than 1 h, without using specialised equipment.

3.3. Effect of bacteria incubation time

Since our assay detects nucleases as a bacterial biomarker rather than the whole bacteria, it was critical to define the minimum incubation interval required to obtain detectable secreted nucleases yielding

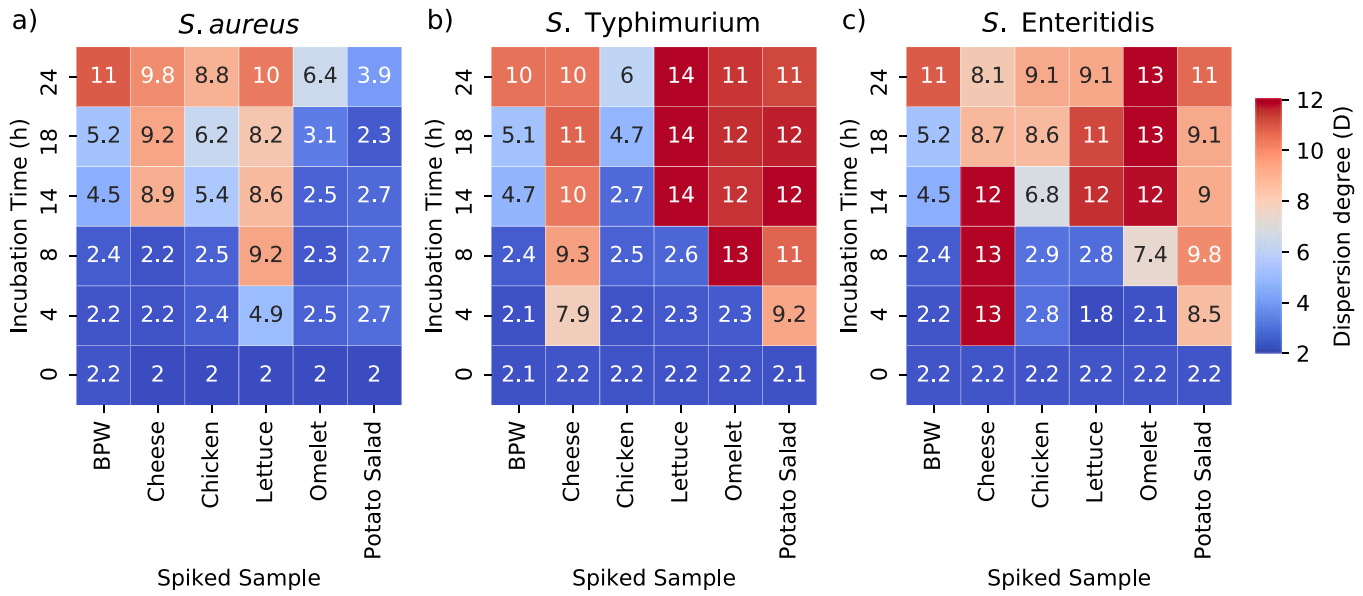

Fig. 5. Detection of bacterial nucleases at different incubation intervals in food samples spiked with *S. aureus*, *S.* Typhimurium or *S.* Enteritidis. Ready-to-eat products such as cheese, chicken, lettuce, omelette or potato salad samples (abscissas axis) were contaminated with 10^3 CFU of strains (a) *S. aureus* 1, (b) *S.* Typhimurium ATCC 14028 or (c) *S.* Enteritidis ATCC 49214, using BPW as control; the supernatants were obtained at 0, 4, 8, 14, 18 and 24 h post-incubation at 37 ºC (ordinates axis), sterilised by filtration and mixed with the duplex linkers composed by (a) Linker A-DNA and Linker B-2′OMe or (b-c) Linker C-RNA and Linker D-2′OMe. Results are expressed as the dispersion degree value (threshold of positivity at D > 5).

positive readouts. Thus, we tested the presence of bacterial nucleases in supernatants of 10^3 to < 10 CFU/mL of *S.* Typhimurium over the time interval ranging from 0 to 24 h (Fig. 4). Nucleases were detected at 10 h of incubation in supernatants containing at least 1000 CFU/mL (Fig. 4a). Decreasing the CFU/mL shifted the detection threshold towards longer incubation time (dashed vertical lines in Fig. 4a-d). To put in context the performance of the plasmonic sensor we evaluated the presence of nucleases for each incubation time using fluorescence-based assay that we have reported recently [13]. As expected, the transition from negative to positive readout falls at the same bacteria incubation interval for both type of sensors (blue-plasmonic assay and grey-fluorescence assay), indicating the optical readout based on aggregation of nanoparticles is a promising strategy for novel colorimetric sensors. Interestingly, the transition threshold from negative to positive readout for both sensors falls at the transition from log to stationary phase of bacterial colony, indicating that nucleases secretion is related to the phase state of a given bacterial colony.

To assess whether nucleases could be detected without bacterial incubation, as previously proposed [13], we checked nanoparticles aggregation for increasing concentrations from 10^1 to 10^8 CFU/mL of the *S. aureus* or *S.* Typhimurium prepared in BPW. As result, all the inocula showed negative results (Fig. S6), suggesting the necessity of an incubation to allow accumulation of detectable nucleases in the medium.

We estimated the nucleases limit of detection (LOD) of the method, by a standard calibration curve with a commercial *S. aureus* micrococcal nuclease. As result, we determined that the method can detect 12.7 μUI/μL, which was equivalent to a D = 1.8 (Fig. S7). This LOD was much lower than the qualitative colorimetric limit (D = 5).

3.4. Nuclease detection in spiked and naturally-contaminated food samples

3.4.1. Spiked food samples

The popularity of ready-to-eat products, not requiring additional preparation prior to consumption, has increased substantially in recent years. In order to assess the utility of our detection method on real samples, we tested ready-to-eat spiked food samples (chicken, lettuce, omelette, potato salad and cottage cheese) by injecting ≈ 10^3 CFU/mL

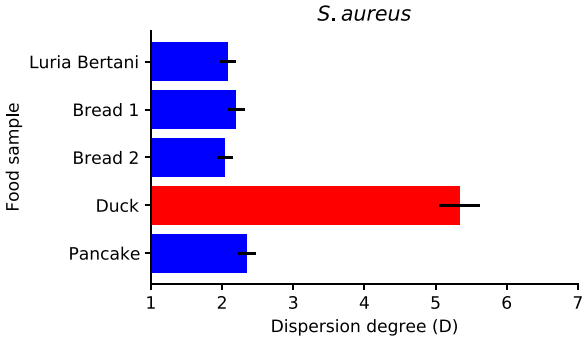

Fig. 6. Detection of *S. aureus* nucleases in commercially-available products. *S. aureus* nucleases were detected in one duck sample naturally contaminated with the pathogen. Bread dough and pancake products resulted naturally contaminated with *S. epidermidis*.

of *S. aureus*, *S.* Typhimurium and *S.* Enteritidis. The supernatants were collected at different incubation times (i.e., 0, 4, 8, 14, 18, and 24 h, at 37 ºC). Interestingly, we observed that the incubation time required for the assay depends on the type of food and the pathogen. For *S. aureus*, the spiked food samples of cheese, chicken and lettuce showed nucleases at shorter incubation times (4 and 14 h) than omelette (24 h), while in potato salad no nucleases were detected over the entire incubation time (Fig. 5a). On the other hand, *S.* Typhimurium and *S.* Enteritidis were detected in cheese and potato salad samples as soon as at 4 h post-incubation, while 8, 14 and 18–24 h were required to detect these pathogens in omelette, lettuce, and chicken samples, respectively (Figs. 5b and 5c). Differences of positive readouts for different food samples regarding the incubation times seemed to stem from the type of nutrients influencing the bacterial growth, nucleases activity and/or nucleases persistence, hence, affecting the final concentration of nucleases.

3.4.2. Naturally-contaminated food samples

To demonstrate the utility of our assay in real-work conditions, we determined the presence of nucleases in four commercially-available

food samples (bread dough, duck and one sample of pancake) that were detected as positive to *Staphylococcus* spp. at the Spanish National Centre of Food Technology (CNTA) using the VIDAS® method (Biomerieux). The samples were processed in a double-blind experiment, revealing the presence of *S. aureus* nucleases in duck product (Fig. 6). The retrospective assessment at the Spanish National Reference Centre Instituto de Salud Carlos III confirmed that the duck sample was indeed the only one contaminated by *S. aureus*, while the rest of samples contained *Staphylococcus epidermidis*.

4. Conclusions

We showed a fast, colorimetric assay able to detect bacterial nucleases by using aggregating nanoparticles as optical signal transducer. In presence of the living pathogen producing the specific nuclease, the probe comprising two complementary oligonucleotide linkers suffers fragmentation, preventing aggregation of nanoparticles, thereby giving a red colour of the solution as a positive outcome. We demonstrated the performance of our assay for nucleases secreted from *S. aureus*, *S.* Typhimurium and *S.* Enteritidis, at a LOD of 1 CFU/mL and 12 μUI/μL of nucleases. We could detect nucleases in both experimentally and naturally contaminated food samples, by naked-eye inspection, in less than two hours.

Our method lays the practical foundation for a nuclease-related test in food samples of many types. A single test's estimated cost of our method is more cost efficient than the current techniques available. Also, since the execution of above-described experiments requires minimal laboratory equipment (i.e. a micropipette and a thermostat), a parallelized testing through automation can be easily implemented. Lastly, techniques currently used in food-safety national centres are designed by a manufacturer to detect specific pathogens. Our method, by featuring a modular character of oligonucleotide probe architecture, offers on-site optimisation of sensor selectivity and specificity through the right combination of linker length, composition, and chemical modifications.

From a broader perspective, we envision that our method, apart from the relevance in food safety-related sector, has the potential to be implemented in other fields such as human and veterinary diagnosis of different bacterial and viral infectious diseases, including COVID-19.

CRediT authorship contribution statement

María Sanromán-Iglesias: Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Victoria Garrido**: Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Yolanda Gil**: Investigation. **Javier Aizpurua:** Conceptualization, Supervision, Writing – review & editing. **Marek Grzelczak**: Conceptualization, Supervision, Project administration, Writing – original draft, Writing – review & editing**.** **María Jesús Grilló**: Conceptualization, Supervision, Project administration, Funding acquisition, Writing - original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2021.130780.

References

[1] B. Stephen Inbaraj, B.H. Chen, Nanomaterial-based sensors for detection of foodborne bacterial pathogens and toxins as well as pork adulteration in meat products, J. Food Drug Anal. 24 (2016) 15–28, https://doi.org/10.1016/j.jfda.2015.05.001.

[2] J.A. Crump, M. Sjolund-Karlsson, M.A. Gordon, C.M. Parry, Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive salmonella infections, Clin. Microbiol. Rev. 28 (2015) 901–937, https://10.1128/CMR.00002-15.

[3] H.L. Alakomi, M. Saarela, Salmonella importance and current status of detection and surveillance methods, Qual. Assur. Saf. Crops Foods 1 (2009) 142–152, https://doi.org/10.1111/j.1757-837X.2009.00032.x.

[4] R.L. Bell, K.G. Jarvis, A.R. Ottesen, M.A. McFarland, E.W. Brown, Recent and emerging innovations in Salmonella detection: a food and environmental perspective, Microb. Biotechnol. 9 (2016) 279–292, https://doi.org/10.1111/1751-7915.12359.

[5] European Comission (EC, Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs Off J Eur Union, L 338 (2005). https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2005:338:TOC. (accessed 25 March 2021).

[6] EFSA-ECDC, The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017, EFSA J. 16 (2018) 5500, https://doi.org/10.2903/j.efsa.2018.5500.

[7] S.M. Pires, L. de Knegt, T. Hald, Estimation of the relative contribution of different food and animal sources to human Salmonella infections in the European Union, EFSA Support. Pub., 8(2011) 184E. https://doi.org/10.2903/sp.efsa.2011.EN-184.

[8] E. Scallan, R.M. Hoekstra, F.J. Angulo, R.V. Tauxe, M.A. Widdowson, S.L. Roy, J. L. Jones, P.M. Griffin, Foodborne illness acquired in the United States-major pathogens, Emerg. Infect. Dis. 17 (2011) 7–15, 10.3201/eid1701.p11101.

[9] ISO, International Organisation for Standardisation. ISO 6579:2002/Amd. 1:2007. Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Detection of Salmonella spp. Amendment 1 Annex D: Detection of Salmonella spp. in Animal Faeces and in Samples from the Primary Production Stage. Geneve, Switzerland. http://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/05/67/56712.html. (accessed 25 March 2021).

[10] R.C. Mainar-Jaime, S. Andres, J.P. Vico, B. San Roman, V. Garrido, M.J. Grillo, Sensitivity of the ISO 6579:2002/Amd 1:2007 standard method for detection of Salmonella spp. on mesenteric lymph nodes from slaughter pigs, J. Clin. Microbiol. 51 (2013) 89–94, https://doi.org/10.1128/JCM.02099-12.

[11] J.W. Law, N.S. Ab Mutalib, K.G. Chan, L.H. Lee, Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations, Front. Microbiol. 5 (2014) 770.

[12] P. Mandal, A. Biswas, K. Choi, U. Pal, Methods for rapid detection of foodborne pathogens: an over-view, Am. J. Food Tech. 6 (2011) 87–102, https://doi.org/10.3389/fmicb.2014.00770.

[13] I. Machado, V. Garrido, L.I. Hernandez, J. Botero, N. Bastida, B. San-Roman, M. J. Grilló, F.J. Hernandez, Rapid and specific detection of Salmonella infections using chemically modified nucleic acid probes, Anal. Chim. Acta 1054 (2019) 157–166, https://doi.org/10.1016/j.aca.2018.12.027.

[14] F.J. Hernandez, L. Huang, M.E. Olson, K.M. Powers, L.I. Hernandez, D. K. Meyerholz, D.R. Thedens, M.A. Behlke, A.R. Horswill, J.O. McNamara, Noninvasive imaging of Staphylococcus aureus infections with a nuclease-activated probe, Nat. Med. 20 (2014) 301–306, https://doi.org/10.1038/nm.3460.

[15] K.J. Lee, W.S. Lee, A. Hwang, J. Moon, T. Kang, K. Park, J. Jeong, Simple and rapid detection of bacteria using a nuclease-responsive DNA probe, Analyst 143 (2017) 332–338, https://doi.org/10.1039/c7an01384a.

[16] E. Mozioglu, S. Akyurek, S. Gunduz, M. Akgoz, A.C. Goren, T. Kocagoz, Oligomer based real-time detection of microorganisms producing nuclease enzymes, Analyst 144 (2019) 1379–1385, https://doi.org/10.1039/c7an01384a.

[17] K. Park, J. Jeong, S.Y. Yi, W.S. Lee, Y.B. Shin, FRET probe-based antibacterial susceptibility testing (F-AST) by detection of bacterial nucleases released by antibiotic-induced lysis, Biosens. Bioelectron. 130 (2019) 225–229, https://doi.org/10.1016/j.bios.2019.01.033.

[18] S.S. Agasti, S. Rana, M.H. Park, C.K. Kim, C.C. You, V.M. Rotello, Nanoparticles for detection and diagnosis, Adv. Drug Deliv. Rev. 62 (2010) 316–328, https://doi.org/10.1016/j.addr.2009.11.004.

[19] H.M. Azzazy, M.M. Mansour, T.M. Samir, R. Franco, Gold nanoparticles in the clinical laboratory: principles of preparation and applications, Clin. Chem. Lab. Med. 50 (2011) 193–209, https://doi.org/10.1515/CCLM.2011.732.

[20] U.H. Bunz, V.M. Rotello, Gold nanoparticle-fluorophore complexes: sensitive and discerning "noses" for biosystems sensing, Angew. Chem. 49 (2010) 3268–3279, https://doi.org/10.1002/anie.200906928.

[21] V.K. Khanna, Nanoparticle-based sensors, Def. Sci. 58 (2008) 608–616.

[22] K. Saha, S.S. Agasti, C. Kim, X. Li, V.M. Rotello, Gold nanoparticles in chemical and biological sensing, Chem. Rev. 112 (2012) 2739–2779, https://doi.org/10.1021/cr2001178.

[23] P. Tallury, A. Malhotra, L.M. Byrne, S. Santra, Nanobioimaging and sensing of infectious diseases, Adv. Drug Deliv. Rev. 62 (2010) 424–437, https://doi.org/10.1016/j.addr.2009.11.014.
[24] V.K. Upadhyayula, Functionalized gold nanoparticle supported sensory mechanisms applied in detection of chemical and biological threat agents: a review, Anal. Chim. Acta 715 (2012) 1–18, https://doi.org/10.1016/j.aca.2011.12.008.
[25] X. Ma, L. Song, N. Zhou, Y. Xia, Z. Wang, A novel aptasensor for the colorimetric detection of S. Typhimurium based on gold nanoparticles, Int. J. Food Microbiol. 245 (2017) 1–5, https://doi.org/10.1016/j.ijfoodmicro.2016.12.024.
[26] S.M. Yoo, D.K. Kim, S.Y. Lee, Aptamer-functionalized localized surface plasmon resonance sensor for the multiplexed detection of different bacterial species, Talanta 132 (2015) 112–117, https://doi.org/10.1016/j.talanta.2014.09.003.
[27] S. Srinivasan, V. Ranganathan, M.C. DeRosa, B.M. Murari, Label-free aptasensors based on fluorescent screening assays for the detection of Salmonella Typhimurium, Anal. Biochem. 559 (2018) 17–23, https://doi.org/10.1016/j.ab.2018.08.002.
[28] X. Wang, S. Ying, X. Wei, J. Yuan, Development of a gold nanoparticle-based universal oligonucleotide microarray for multiplex and low-cost detection of foodborne pathogens, Int. J. Food Microbiol. 253 (2017) 66–74, https://doi.org/10.1016/j.ijfoodmicro.2017.05.005.
[29] L. Zheng, G. Cai, S. Wang, M. Liao, Y. Li, J. Lin, A microfluidic colorimetric biosensor for rapid detection of Escherichia coli O157:H7 using gold nanoparticle aggregation and smart phone imaging, Biosens. Bioelectron. 124–125 (2019) 143–149, https://doi.org/10.1016/j.bios.2018.10.006.
[30] A.S. Afonso, B. Pérez-López, R.C. Faria, L.H. Mattoso, M. Hernández-Herrero, A. X. Roig-Sagués, M.Maltez-da Costa, A. Merkoçi, Electrochemical detection of Salmonella using gold nanoparticles, Biosens. Bioelectron. 40 (2013) 121–126, https://doi.org/10.1016/j.bios.2012.06.054.
[31] T.R. de Oliveira, D.H. Martucci, R.C. Faria, Simple disposable microfluidic device for Salmonella Typhimurium detection by magneto-immunoassay, Sens. Actuators B Chem. 255 (2018) 684–691, https://doi.org/10.1016/j.snb.2017.08.075.
[32] N.A. Masdor, Z. Altintas, I.E. Tothill, Sensitive detection of Campylobacter jejuni using nanoparticles enhanced QCM sensor, Biosens. Bioelectron. 78 (2016) 328–336, https://doi.org/10.1016/j.bios.2015.11.033.
[33] F. Salam, Y. Uludag, I.E. Tothill, Real-time and sensitive detection of Salmonella Typhimurium using an automated quartz crystal microbalance (QCM) instrument with nanoparticles amplification, Talanta 115 (2013) 761–767, https://doi.org/10.1016/j.talanta.2013.06.034.
[34] N.G. Bastus, J. Comenge, V. Puntes, Kinetically controlled seeded growth synthesis of citrate-stabilized gold nanoparticles of up to 200 nm: size focusing versus Ostwald ripening, Langmuir 27 (2011) 11098–11105, https://doi.org/10.1021/la201938u.
[35] S.J. Hurst, A.K. Lytton-Jean, C.A. Mirkin, Maximizing DNA loading on a range of gold nanoparticle sizes, Anal. Chem. 78 (2006) 8313–8318, https://doi.org/10.1021/ac0613582.

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