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Electrochemical genosensing of *Salmonella*, *Listeria* and *Escherichia coli* on silica magnetic particles

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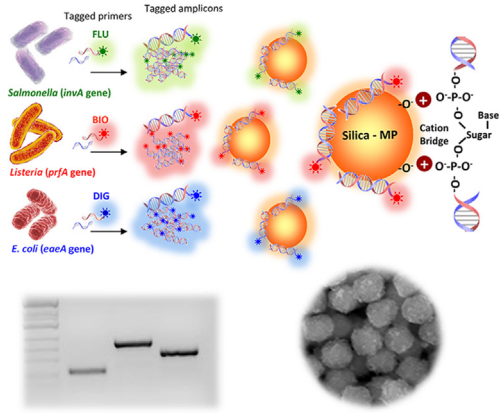
HIGHLIGHTS

- Silica magnetic particles were used for the first time as carrier in electrochemical magneto-genosensing of single-tagged amplicons.
- They demonstrated to be a robust platform for the electrochemical detection of PCR products.
- Differential adsorption properties for longer dsDNA amplicon incorporating the tagging primers over shorter ssDNA tagged primers were observed due to the negative charge density.
- Electrochemical magneto-genosensing of *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli* was successfully performed.

GRAPHICAL ABSTRACT



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ABSTRACT

A magneto-genosensing approach for the detection of the three most common pathogenic bacteria in food safety, such as *Salmonella, Listeria* and *Escherichia coli* is presented. The methodology is based on the detection of the tagged amplified DNA obtained by single-tagging PCR with a set of specific primers for each pathogen, followed by electrochemical magneto-genosensing on silica magnetic particles. A set of primers were selected for the amplification of the *invA* (278 bp), *prfA* (217 bp) and *eaeA* (151 bp) being one of the primers for each set tagged with fluorescein, biotin and digoxigenin coding for *Salmonella enterica, Listeria monocytogenes* and *E. coli*, respectively. The single-tagged amplicons were then immobilized on silica MPs based on the nucleic acid-binding properties of silica particles in the presence of the chaotropic agent as guanidinium thiocyanate. The assessment of the silica MPs as a platform for electrochemical magneto-genosensing is described, including the main parameters to selectively attach longer dsDNA fragments instead of shorter ssDNA primers based on their negative charge density of the sugar-phosphate backbone. This approach resulted to be a promising detection tool with sensing features of rapidity and sensitivity very suitable to be implemented on DNA biosensors and microfluidic platforms.

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1. Introduction

Foodborne illnesses caused by microorganisms, among them bacteria, are a large, widespread and growing public health problem. Although food safety practices are being improved due to severe regulatory actions, there remains a growing need for enhanced rapid tools for food pathogen detection. The last outbreak of *Escherichia coli* Shiga toxin-producing O104:H4 in Europe in 2011 is only one example, demonstrating the difficulties in promptly detecting contaminations and avoiding their spread [1]. Conventional assays in common use are based on microbiological techniques. Even though these methods are still effective for the recognition of microorganisms, they are rather time-consuming requiring several days for completion. In this regards, polymerase chain reaction (PCR) has been widely considered a rapid and sensitive method for the detection and identification of bacteria. Particularly, the current trend is moving towards strategies for a rapid identification of more than one pathogen through the development of multiple analysis platforms [2–5]. Multiple pathogen detection represents an option to decrease reagent costs and speed up detection of several pathogens in one reaction. Generally, the most studied are the six pathogens that account for over 90% of estimated food-related deaths: *Salmonella* (31%), *Listeria* (28%), *Toxoplasma* (21%), Norwalk-like viruses (7%), *Campylobacter* (5%), and *E. coli* O157:H7 (3%) [6]. To address this issue, multiplexed PCR [7] and both DNA [8,9] and antibody [10] microarray techniques have been developed to identify from two to five foodborne pathogens in one assay. Biosensors have been recently improved as well for the multiplex detection of pathogens by the implementation of nanomaterials such as quantum dots, gold nanorods and silver or gold nanoparticles [1,11,and12]. Magnetic particles deserve among all nanomaterials, a special mention for their wide range of applications across many biological fields, for the immunomagnetic separation (IMS), as a replacement of the selective plating [13–15] and for the magnetic capture hybridization (MCH) to separate pathogen DNA from inhibitory compounds before PCR amplification [3,16and17]. Magnetic particles can be also used as a detection label by using magneto-resistance sensors [18,19]. Particularly, silica magnetic particles (silica MPs) have been widely used due to the variety of chemical and physical modifications, and their biocompatibility [20]. Silica particles were extensively used for the isolation of PCR products and genomic DNAs [21,22]. DNA interaction with silica was studied by Melzak et al. [23] being attributed to the adsorption of highly charged duplex DNA to hydrophilic negatively charged silica. The DNA adsorption is controlled by three competing effects: weak electrostatic repulsion forces, dehydration and hydrogen bond formation. Although the mechanism is not fully understood, the high affinity between DNA and silica has attracted significant attentions. Kits and materials for DNA extraction involving the use of silica particles are available from many companies. The vast majority of them comprise only the binding, washing and elution of DNA steps allowing downstream applications to user requirements.

This paper addresses the novel application of silica MPs as a platform for electrochemical magneto-genosensing, an additional use from the known extraction, purification and further elution of nucleic acids. This approach was performed by the release of the bacteria genome of three of the most studied pathogens, *Salmonella, Listeria* and *E. coli*, followed by PCR in order to obtain the single-tagged amplicons by using fluorescein, biotin and digoxigenin as coding tags for one of the primers of each set. The tagging primers were selected for the specific amplification of the *invA* (278 bp), *prfA* (217 bp) and *eaeA* (151 bp) genes related to *Salmonella enterica*, *Listeria monocytogenes* and *E. coli* respectively. The amplicons, tagged with fluorescein, biotin and digoxigenin for *S. enterica*, *L. monocytogenes* and *E. coli* respectively, were then immobilized on silica MPs. To confirm the identity of the three bacteria, the single-tagged amplicons were detected by electrochemical magneto-genosensing using three different electrochemical reporters, AntiFluorescein-HRP, Streptavidin-HRP and AntiDigoxigenin-HRP conjugates respectively. The main parameters for the attachment, based on their negative charge density of the sugar-phosphate backbone, of longer dsDNA amplicons instead of shorter ssDNA primers were extensively studied. The main features of this approach are discussed for implementation on multiplex tagging PCR approaches and on microfluidic systems with electrochemical detection mainly for food industry application.

2. Materials and methods

2.1. Instrumentation

The PCR reaction was carried out in a thermal cycler (Product N° 2720, Applied Biosystems, Life Technologies Corporation). Temperature-controlled incubations in Eppendorf tubes were performed in an Eppendorf Thermomixer compact. The magnetic separation was performed using a magnetic separator Dynal MPC-S (Product N° 120.20D, Dynal Biotech ASA, Norway). Amperometric measurements were performed with a LC-4C amperometric controller (BAS Bioanalytical Systems Inc, USA). A three-electrode setup was used comprising a platinum auxiliary electrode (Crison 52-67 1), a double junction Ag/AgCl reference electrode (Orion 900200) with 0.1 mol L^{-1} KCl as the external reference solution and a working electrode (the magneto electrode, m-GEC). The detailed preparation of the m-GEC electrodes was extensively described by Pividori et al. [24,25]. The SEM images were taken with the scanning electron microscope Hitachi LTD S-570 (Hitachi LTD, Tokyo, Japan).

2.2. Chemicals and biochemicals

The Expand High Fidelity PCR System Kit (Roche Molecular Biochemicals) was used for performing the PCR. The primers for the PCR amplification in the genosensing strategies were obtained from Roche Diagnostics S. L. Dynabeads SILANE genomic DNA kit (Product N° 370.12D) and silica magnetic particles (silica MPs) (Dynabeads MyOne Silane, Product N° 37002D) were purchased from Life Technologies, Invitrogen Dynal AS. AntiFlu-HRP (Anti-Fluorescein-Fab fragments, 11426346910), Strep-HRP (Streptavidin-POD conjugate, 1089153001) and AntiDig-HRP (Anti-Digoxigenin-POD Fab fragments, 11207733910) were purchased from Roche Diagnostics S. L. All buffer solutions were prepared with milli-Q water and all other reagents were in analytical reagent grade (supplied from Sigma and Merck). The composition of these solutions were: Binding buffer (0.1 mol L^{-1} Tris, 5 mol L^{-1} GuSCN, pH 6.4); Triton-binding buffer (0.1 mol L^{-1} Tris, 5 mol L^{-1} GuSCN, 0.1 mol L^{-1} Triton X-100, 40 mmol L^{-1} EDTA, pH 6.4); Washing buffer (10 mmol L^{-1} Tris, pH 4.5); Tris buffer (0.1 mol L^{-1} Tris, 0.15 mol L^{-1} NaCl, pH 7.5); Blocking Tris buffer (2% w/v BSA, 0.1% w/v Tween 20, 5 mmol L^{-1} EDTA, in Tris buffer); Phosphate buffer (0.1 mol L^{-1} sodium phosphate, 0.1 mol L^{-1} KCl, pH 7.0).

2.3. Bacterial strains, growth conditions and DNA extraction

S. enterica Typhimurium LT2, *L. monocytogenes* DSM20600 (DSMZ) and *E. coli* K12 strains were used in this work. All bacterial strains were grown in Luria Bertani (LB) broth or agar plates for 18 h at 37 °C.

The lysis of the bacteria and the DNA extraction and purification were performed according to the kit manufacturer (DNeasy Tissue