



Fast detection of *Salmonella* Infantis with carbon nanotube field effect transistors

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ABSTRACT

In this paper we report a fast, sensitive and label-free biosensor for the selective determination of *Salmonella* Infantis. It is based on a field effect transistor (FET) in which a network of single-walled carbon nanotubes (SWCNTs) acts as the conductor channel. Anti-*Salmonella* antibodies were adsorbed onto the SWCNTs and subsequently the SWCNTs were protected with Tween 20 to prevent the non-specific binding of other bacteria or proteins. Our FET devices were exposed to increasing concentrations of *S. Infantis* and were able to detect at least 100 cfu/mL in 1 h. To evaluate the selectivity of our FET devices, *Streptococcus pyogenes* and *Shigella sonnei* were tested as potential competing bacteria for *Salmonella*. At a concentration of 500 cfu/mL, neither *Streptococcus* nor *Shigella* interfered with the detection of *Salmonella*. Therefore, these devices could be used as useful label-free platforms to detect *S. Infantis* and, by using the suitable antibody, other bacteria or viruses.

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1. Introduction

Salmonella enterica subsp. *enterica* serotype Infantis (*S. Infantis*) is a rod-shaped bacterium that is found in the bacterial flora of living reptiles and amphibians. It is also very common in poultry, red meat, raw egg shells, unpasteurized milk and their dairy products. *S. Infantis* can cause gastroenteritis and can be transmitted through the ingestion of contaminated food or water (Boyle et al., 2007; Plym and Wierup, 2006; Shahada et al., 2006). It is one of the most significant pathogenic microorganisms in food-borne infections in humans due to its multidrug resistance. For this reason, there is a widespread need to protect public health by developing fast and reliable techniques to detect it at low concentrations in food and water.

Conventional culturing methods are reliable but very time consuming (i.e. typically requiring 3–7 days) and other methods such as the polymerase chain reaction (PCR) or enzyme-linked immunosorbent assays (ELISA) require between 8 and 48 h. Some biosensors have been developed for the label-free detection of

pathogens, although they still have limits of detection (LODs) of about 10³ cfu/mL (Taylor et al., 2006).

Single-walled carbon nanotubes (SWCNTs) have outstanding electrical and mechanical properties. Tans et al. (1998) reported the first field effect transistor (FET)-based on a single SWCNT and Kong et al. (2000) used it to develop the first carbon nanotube field effect transistor (CNTFET) chemical sensor. The functionalization of SWCNTs gives rise to the development of selective CNTFET biosensors based on the principles of molecular recognition. Star et al. used a CNTFET based on a single SWCNT to detect streptavidin by means of the biotin–streptavidin interaction (Star et al., 2003). Byon et al. detected protein–protein interactions at 1 pM concentration with a highly sensitive FET based on networks of SWCNTs (Byon and Choi, 2006). CNTFETs functionalized with aptamers have been applied to detect either thrombin (So et al., 2005) or immunoglobulin E (Maehashi et al., 2006).

So far CNTFETs have not been applied to detect bacteria or higher organisms. Only the interaction between CNTs and bacteria has already been studied. Huang et al. (2004) adsorbed anti-*Salmonella* antibodies on CNTs and then exposed it to 10⁸ cfu/mL of *Salmonella typhimurium* for 1 h. The bacteria linked to the CNTs were observed with scanning electron microscopy (SEM). Elkin et al. (2005) prepared immuno-carbon nanotubes by functionalizing SWCNTs with albumin serum bovine (BSA) and anti-*E. coli* antibodies. The interaction between the immuno-SWNTs and *E. coli* cells was evidenced

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both by SEM and the use of a green fluorescent protein secondary antibody as a marker. Lin et al. (2006) used anti-*E. coli* antibodies to functionalize multi-walled carbon nanotubes (MWCNTs) with encapsulated ferromagnetic elements. After the immunomagnetic separation of the MWCNTs, they were able to selectively detect 400 cfu/mL of *E. coli*.

The antigen–antibody interaction implemented as the sensing layer together with the capability of CNTs to transduce the charge transfer by using FETs can be further exploited to detect cells. In this study, we report the development of fast and sensitive CNT-FET to selectively detect at least 100 cfu/mL of *S. Infantis* in 1 h. The recognition layer of the CNTFET consists of the anti-*Salmonella* antibodies adsorbed onto the CNTs. Tween 20 was used to avoid the non-specific binding (NSB) of other bacteria or proteins. The molecular recognition mechanism used and the ability of the CNTs to transduce the presence of *S. Infantis* makes labels unnecessary.

2. Experimental

2.1. Materials

Anti-*Salmonella* (i.e. a rabbit polyclonal antibody for *Salmonella* “O” & “H” antigens (4–5 mg/mL)), was purchased from Oxford Biotechnology Ltd. (Oxford, UK). It was dissolved in phosphate buffer saline (PBS) to a final concentration of 10 ppm (pH 7.2) and stored at -20°C until use. Tween 20 and PBS were obtained from Sigma–Aldrich. Trypticase soy agar (TSA) and trypticase soy broth (TSB) were provided by Beckton–Dickinson and prepared according to their specifications. Silver ink was provided by Acheson industries.

2.2. Bacterial preparation

Stocks of strains of *S. Infantis* S34/11, *Shigella sonnei* (*S. sonnei*) CECT 413 and *Streptococcus pyogenes* (*S. pyogenes*) CECT 985T were obtained from the Faculty of Medicine and Health Sciences at the Rovira i Virgili University. They were stored at -20°C with glycerol. *S. Infantis* was reactivated by inoculating the bacteria in 10 mL of sterile TSB at 37°C for 12 h. Then, the sample was pelletized in a centrifuge at 6000 rpm for 15 min and the supernatant was discarded. The pellet was resuspended in 10 mL of sterile 0.85% saline solution. The bacteria contained in the saline solution were enumerated by plating sevenfold serial dilutions on TSA plates and incubating at 37°C for 24–48 h. Further dilutions in sterile 0.85% saline solution were made to produce the final samples at 100, 300 and 500 cfu/mL of *S. Infantis*. The same procedure was followed to prepare 500 cfu/mL of *S. sonnei* and *S. pyogenes*.

2.3. Apparatus

Atomic force microscopy (AFM), Pico Plus (Molecular Imaging) in tapping mode was used to obtain the images of the networks of CNTs. Scanning electron microscopy (SEM), JSM 6400 (Jeol) was used to obtain the images of the functionalization process of the CNTs. SEM images were obtained by applying a voltage of 5 kV at a working distance of 8 mm. Electrical measurements were made using a 4157A Agilent semiconductor parameter analyzer and a Wentworth Laboratories MP1008 probe station.

2.4. Development of the CNTFETs

The CNTs networks were synthesized on top of a 500 nm layer of silicon dioxide thermally grown on highly n-type doped silicon chips (total area $0.5\text{ cm} \times 0.5\text{ cm}$) by chemical vapour deposition (CVD). A solution of 100 ppm iron nitrate in isopropanol was used

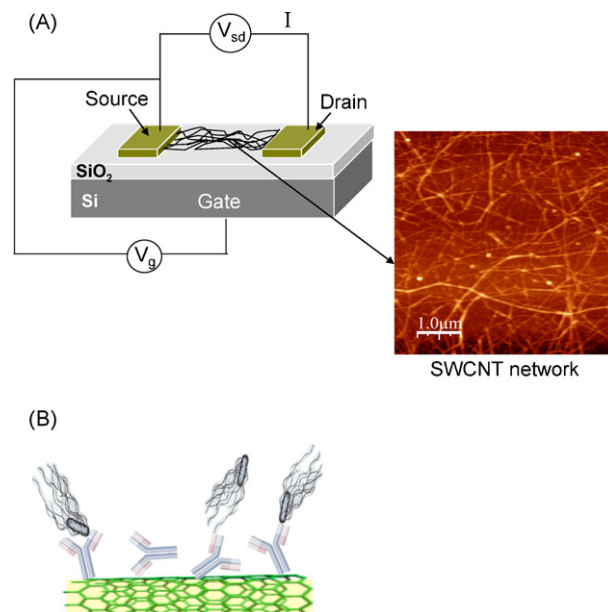


Fig. 1. (A) Experimental set up for detecting *Salmonella Infantis* with a network of CNTFETs functionalized with anti-*Salmonella* antibodies. (B) Antigen–antibody interaction of *S. Infantis* with a SWCNT functionalized with anti-*Salmonella* antibodies and protected with Tween 20.

as the catalyst. The CVD was performed at 900°C for 20 min with 600 sccm of methane and 200 sccm of hydrogen. AFM (Fig. 1A) shows that a dense network of SWCNTs with an average height of $\sim 1.5\text{ nm}$ was obtained after the CVD. The source and drain electrodes of the CNTFETs were screen-printed with silver ink. The gap between both electrodes was 0.5 mm and the size of the electrodes was $200\text{ }\mu\text{m} \times 200\text{ }\mu\text{m}$. The gate electrode was an aluminium layer on the back side of Si. The CNTFETs were electrically characterized by recording the current vs. the gate voltage. To obtain the instrumental variability, we carried out all the electrical measurements three times and we plotted the mean value and the range of the measurements.

2.5. Functionalization of the CNTFETs

The CNTFETs were functionalized as follows. First, the CNTFETs were incubated for 1 h at 37°C in a 10 ppm solution of anti-*Salmonella* antibodies in PBS solution 15 mM (Lin et al., 2006; Bradley et al., 2004). Then, the CNTFETs were rinsed with PBS solution and distilled water, dried with nitrogen (Ilic et al., 2000) and immersed for 2 h in a solution of 0.5% Tween 20 dissolved in PBS 15 mM (So et al., 2005). The CNTFETs were again thoroughly rinsed with distilled water, dried with nitrogen and ready to be used to detect *S. Infantis*. Fig. 1A shows a scheme of the CNTFET and Fig. 1B shows the interaction of *S. Infantis* with the antibodies adsorbed onto the CNTs.

2.6. Detection of *S. Infantis*

The functionalized CNTFETs were exposed to increasing concentrations of *S. Infantis* (i.e. first to 100 cfu/mL, then to 300 cfu/mL and finally to 500 cfu/mL). For each concentration, the CNTFETs were immersed for 1 h at 37°C , rinsed thoroughly with distilled water, dried with nitrogen and electrically characterized. For each concentration we scanned the area between the electrodes ($\sim 500\text{ }\mu\text{m}^2$) with SEM to count the number of bacteria attached to the CNTs.

2.7. Selectivity of the CNTFETs

Selectivity was checked in the presence of two bacteria: *S. pyogenes* and *S. sonnei*. A functionalized CNTFET was first immersed in a bacteria solution containing 500 cfu/mL of *S. pyogenes* for 1 h at 37 °C, thoroughly rinsed with distilled water, dried with nitrogen and electrically characterized. Subsequently it was exposed to 500 cfu/mL *S. Infantis* under the same conditions mentioned above. This procedure was also followed for 500 cfu/mL of *S. sonnei*. All these experiments were confirmed microscopically with SEM.

3. Results and discussion

The CNTFETs were electrically characterized after each functionalization step in dry conditions by measuring three times the dependence of the source-drain current, I , on the back gate voltage, V_g , in the range +10 to –10 V (Bradley et al., 2004). The bias voltage, V_{sd} , was fixed at 250 mV. Fig. 2 shows how each functionalization step affects the electrical current of a typical CNTFET. Each electrical current plotted corresponds to the mean value and range of the replicates. The range of the measured electrical current was in all cases less than 10% of the electrical current measured. A p-type behaviour was observed for the as grown networks due to the electron withdrawal of adsorbed oxygen molecules from the air (Star et al., 2003).

Anti-*Salmonella* antibodies were adsorbed on the carbon nanotubes. This phenomenon has been attributed to several origins, among them, the size compatibility between the two entities (Shim et al., 2002) and the role of hydrophobic interactions (Chen et al., 2003). We observed through the SEM that 1 h was enough to immobilize anti-*Salmonella* antibodies onto the side walls of CNTs. We took SEM images of a CNTFET just after the antibodies had been immobilized and the CNTFET had been submerged in PBS solution for 24 h. We observed a uniform layer of antibodies, suggesting that they were strongly adsorbed on the CNTs since the density of antibodies adsorbed did not decrease 24 h later. The electrical current of the device decreased after the adsorption of anti-*Salmonella* due to the charge transfer process in which the antibodies provide electrons to the CNTs (Grüner, 2006). These electrons are provided by the amine groups of aminoacids with base containing residues (i.e. arginine, histidine and lysine) that are located at the external envelope of the protein. Each adsorbed amine donates 0.04 electrons to the nanotubes giving rise to a decrease of the electrical current

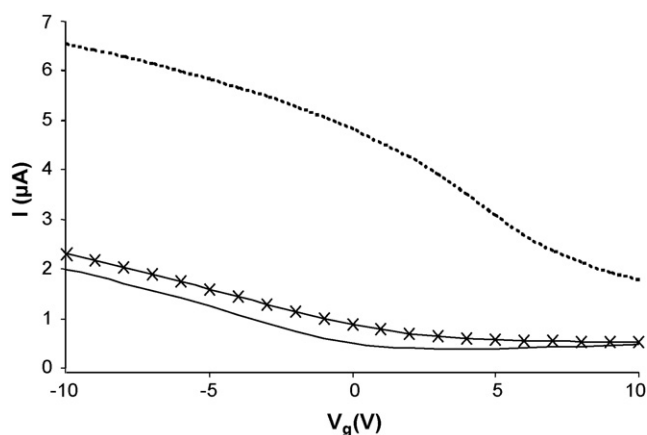


Fig. 2. Gate voltage dependence of the source-drain current of a typical CNTFET after each functionalization step. Pristine CNTs (---); anti-*Salmonella* antibodies adsorbed on the CNTs (x) and CNTs covered with anti-*Salmonella* antibodies and Tween 20 (—).

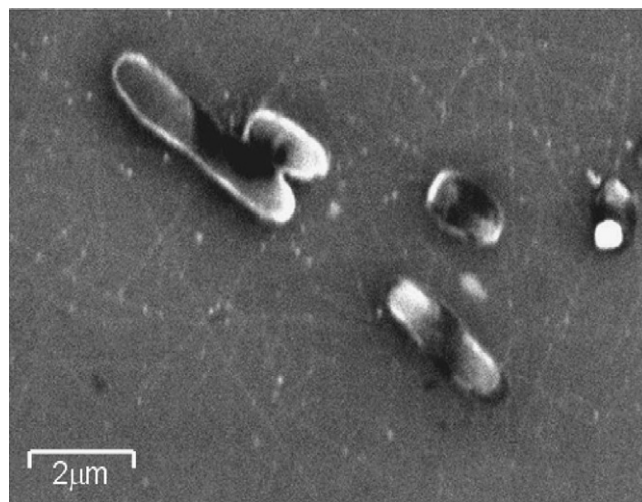


Fig. 3. SEM image of a typical functionalized device after exposure to 500 cfu/mL of bacteria for 1 h at 37 °C. *S. Infantis* is linked to the CNTs network through antigen–antibody interactions. The small dots along the carbon nanotubes, some of them much brighter, are the antibodies adsorbed onto the CNTs.

and to a shift in the threshold voltage of the semiconducting CNTs (Bradley et al., 2004).

The CNTFET electrical current decreased slightly after the adsorption of Tween 20, as previously observed by So et al. (2005). Tween 20 is a surfactant with linear aliphatic chains that adsorbs on the walls of CNTs by hydrophobic interactions and protects them against the NSB of proteins (Byon and Choi, 2006; So et al., 2005; Chen et al., 2003) or bacteria. In our case, Tween 20 covers the gaps of the CNTs walls left unprotected by the adsorbed anti-*Salmonella* protein. These functionalized devices were subsequently exposed to solutions of *S. Infantis* for 1 h at 37 °C. Fig. 3 shows a SEM image of a FET device exposed to *Salmonella*. Notice that the immobilization of anti-*Salmonella* antibodies can be observed as bright dots on the SWCNTs. The bacteria are linked to the CNTs through antigen–antibody interaction.

The functionalized CNTFETs were exposed to increasing concentrations of *S. Infantis*. For each concentration, the CNTFETs were immersed for 1 h at 37 °C, rinsed thoroughly with distilled water, dried with nitrogen and electrically characterized by measuring three times the I – V characteristics and by plotting the mean value and range of the measurement values. Fig. 4 shows the I – V characteristics (for a bias = 0.25 V) of a functionalized CNTFET before exposure to *S. Infantis* and after exposure to 100, 300 and 500 cfu/mL. The inset in Fig. 4 shows that the adsorption of *S. Infantis* significantly decreases the electrical current at negative gate voltages (i.e. from a 45% decrease for 100 cfu/mL to an 80% decrease for 500 cfu/mL). The error bars (that correspond to the range of the electrical current measured for the three replicates) show that the variability of the electrical current was lower than 10%. The decrease of the electrical current is probably due to the antibodies interacting with the somatic O antigen (O-antigen) and the flagellar H antigen (H-antigen) present in the outer membrane and the flagellums of *S. Infantis*, respectively. The O-antigen consists of a large polymer of repeating oligosaccharide units giving the major antigen specificity of the organism, because the monosaccharide units vary among species and even among strains. The H-antigen is composed of a single globular protein, flagellin (Johnson, 1967; Wang et al., 2003). In a similar way as with the adsorption of the antibody, either the charge transfer mainly from the amino groups of the bacterial proteins or the distortion of the CNTs could be the reason for the conductance decrease (Star et al., 2003; Grüner, 2006). The inset

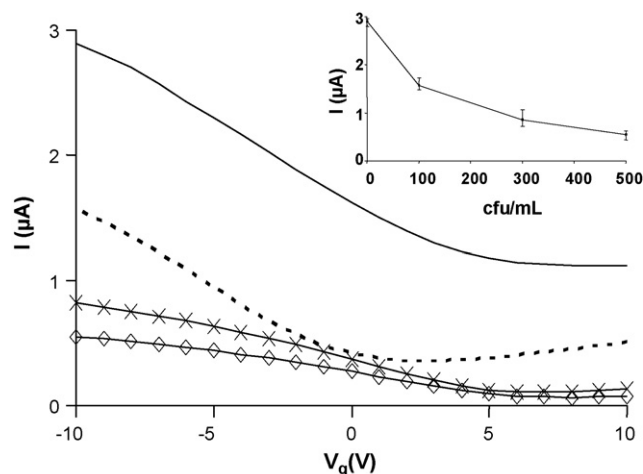


Fig. 4. Gate voltage dependence of the source-drain current of a typical CNTFET before exposure to *S. Infantis* (—) and after exposure to 100 cfu/mL (---); 300 cfu/mL (×) and 500 cfu/mL (◇) of *S. Infantis*. Each electrical current plotted corresponds to the mean value of three replicates. The inset shows the behaviour of the source-drain current vs. increasing bacteria concentration (cfu/mL) at $V_g = -10$ V. The error bars correspond to the range of electrical current measured for the three replicates.

in Fig. 4 shows that the higher the concentration of *Salmonella* is, the more the electrical current decreases. Since the bacteria were prepared in sterile 0.85% NaCl solution, we checked the possible effect of the interferences due to the matrix components, i.e. if the decrease of the electrical current could also be due to the presence of NaCl. As a control experiment, we exposed the devices to a 0.85% NaCl solution for 1 h at 37 °C and no significant change of the electrical current was observed. The measured response of the devices exposed to *Salmonella* was stable for at least 24 h, i.e. the electrical current measured showed no drift and a variability lower than 10%.

SEM confirmed that the number of bacteria attached to the CNTs was proportional to the concentration of *Salmonella* in solution. A functionalized CNTFET was exposed to different bacteria concentrations for 1 h each, thoroughly rinsed with distilled water and dried with nitrogen. For each concentration we scanned the area between the electrodes ($\sim 500 \mu\text{m}^2$) with SEM to count the number of bacteria attached to the CNTs. We counted 25, 36 and 63 bacteria after exposing the CNTFET to 100, 300 and 500 cfu/mL, respectively.

As a control experiment, we submerged CNTFET devices in Tween 20 without having adsorbed the anti-*Salmonella* antibodies on the CNTs. These devices were subsequently exposed to 500 cfu/mL *S. Infantis* for 1 h at 37 °C. Fig. 5 shows that the electrical current changed slightly after the device was exposed to 500 cfu/mL of *S. Infantis*. The electrical current plotted corresponds to the mean value of the three measurements. The error bars of the inset (that correspond to the range of the current values) show that the slight change of the electrical current is due to the variability of the electrical current. In this way we proved that Tween 20 was effectively protecting the CNTs against the NSB of bacteria and that the decrease in the electrical current was only due to antigen–antibody interaction.

The effectiveness of Tween 20 against the NSB was also checked with SEM. A CNTFET (functionalized with Tween 20 but not with anti-*Salmonella*) was exposed to 100, 300 and 500 cfu/mL *S. Infantis* for 1 h each, thoroughly rinsed with distilled water and dried with nitrogen. For each concentration we scanned the area between the electrodes ($\sim 500 \mu\text{m}^2$) with SEM. Only one bacterium was bound when the device was exposed to 100 cfu/mL of *S. Infantis* whereas two bacteria were observed when it was exposed to 300 and 500 cfu/mL of *S. Infantis*.

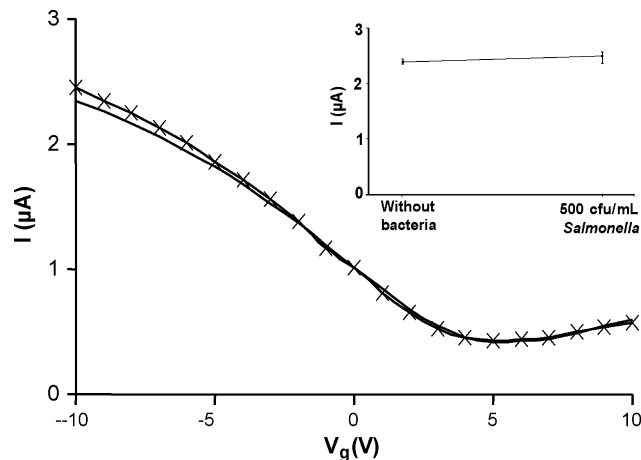


Fig. 5. Electrical behaviour of a Tween 20 modified-CNTFET before exposure to *S. Infantis* (—) and after exposure to 500 cfu/mL (×) of *S. Infantis*. Each electrical current plotted corresponds to the mean value of three replicates. The inset shows the behaviour of the source-drain current at $V_g = -10$ V. The error bars correspond to the range of the electrical current measured for the three replicates.

Selectivity of our sensors devices was checked in the presence of two bacteria: *S. pyogenes* and *S. sonnei*. These pathogens are normal part of animal flora and can be found together with *Salmonella* in contaminated food or water. *S. pyogenes* should not have any cross-reactions with anti-*Salmonella* antibody because it is a Gram-positive bacterium that is totally different from *S. Infantis*. On the other hand, *S. sonnei* is a Gram-negative bacterium that is morphologically and antigenically similar to *S. Infantis*. However, no cross-reaction was observed with *S. sonnei*.

A functionalized CNTFET was first immersed in a bacteria solution containing 500 cfu/mL of *S. pyogenes* for 1 h at 37 °C, thoroughly rinsed with distilled water, dried with nitrogen and electrically characterized. Subsequently it was exposed to 500 cfu/mL *S. Infantis* under the same conditions mentioned above. This procedure was also followed with another functionalized CNTFET for 500 cfu/mL of *S. sonnei*. Fig. 6A and B shows that the electrical current changes slightly after the CNTFETs are exposed either to *S. pyogenes* or *S. sonnei* whereas it decreases between 50 and 60% after exposure to *S. Infantis*. The slight changes of the electrical current after exposing the devices to *S. pyogenes* and *S. sonnei* are due to the variability of the electrical current.

Selectivity was also checked with SEM. A functionalized CNTFET was exposed to 100, 300 and 500 cfu/mL of *S. sonnei* for 1 h each, thoroughly rinsed with water and dried with nitrogen. For each concentration, we scanned the area between the electrodes ($\sim 500 \mu\text{m}^2$) with SEM. We observed only two bacteria bound to the CNTFET after it had been exposed to 100 cfu/mL. No further bacteria were attached after the CNTFET was exposed to 300 and 500 cfu/mL of *S. sonnei*. The same procedure was followed for *S. pyogenes*. In this case, no bacteria were attached to the CNTs after a functionalized CNTFET device was exposed to 100, 300 and 500 cfu/mL *S. pyogenes*. We can conclude that at a concentration of 500 cfu/mL, neither *S. sonnei* nor *S. pyogenes* interfered with the detection of *S. Infantis*. Therefore, Tween 20 avoids the NSB of other bacteria and the antibody has no significant cross-reaction with Gram-negative bacteria that are similar to *S. Infantis*. Our sensor devices are then able to detect selectively at least 100 cfu/mL of *S. Infantis*. This limit of detection is similar to the one obtained for *Salmonella* with other type of recently developed biosensors based on quartz crystal microbalance (Su and Li, 2005), piezoelectric transducers (Olsen et al., 2006) and surface plasmon resonance (Oh et al., 2004).

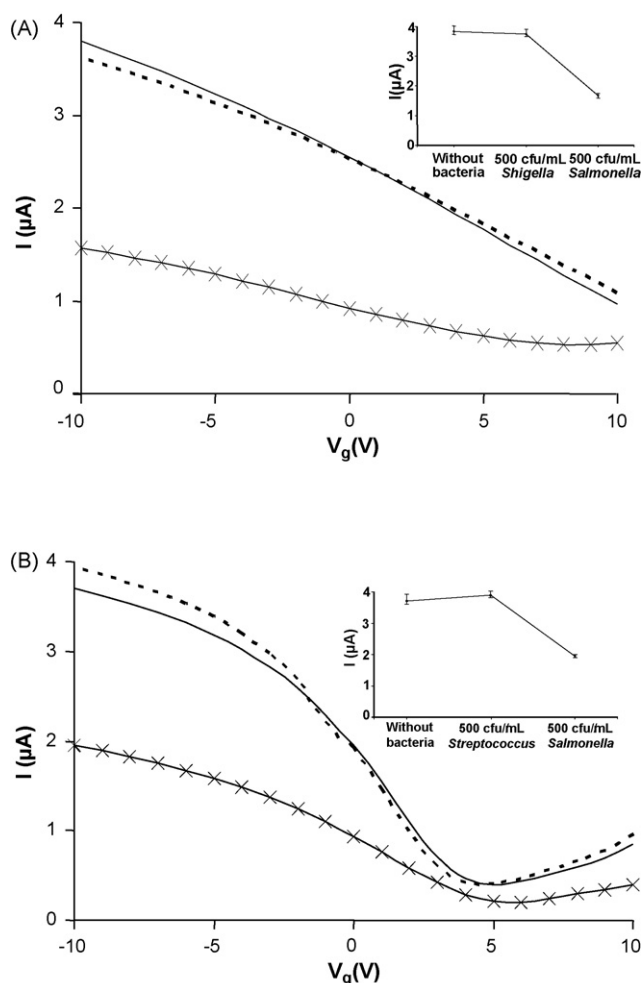


Fig. 6. (A) Gate voltage dependence of the source-drain current of a functionalized CNTFET before exposure to the bacteria (—) and after exposure to 500 cfu/mL of *S. sonnei* (---) and 500 cfu/mL of *S. Infantis* (x). (B) Gate voltage dependence of the source-drain current of a functionalized CNTFET before exposure to the bacteria (—) and after exposure to 500 cfu/mL of *S. pyogenes* (---) and 500 cfu/mL of *S. Infantis* (x). Each electrical current plotted corresponds to the mean value of three replicates. The inset shows the behaviour of the source-drain current at $V_g = -10$ V. The error bars correspond to the range of the electrical current measured for the three replicates.

4. Conclusions

We have developed a fast, sensitive and label-free biosensor for the selective detection of *S. Infantis*. It is based on the transduction

power of CNTFETs combined with the recognition capacity of the antigen–antibody interaction. It can detect at least 100 cfu/mL of *S. Infantis* in just 1 h. This is clearly better than the time responses of the current techniques used for detecting this pathogen.

This biosensor, then, could be used as a useful label-free platform to detect other pathogenic bacteria, viruses or eukaryotic cells like moulds and yeast by using an adequate molecular receptor. The performance of these devices will be evaluated by applying them to other strains of *Salmonella* and also to food products contaminated with *Salmonella*.

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