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OLED-based DNA biochip for *Campylobacter* spp. detection in poultry meat samples

Marisa Manzano [a,*], Francesca Cecchini [a], Marco Fontanot [a], Lucilla Iacumin [a], Giuseppe Comi [a], Patrizia Melpignano [b,c]

[a] Department of Food Science, University of Udine, via Sondrio 2/A, 33100 Udine, Italy
[b] Université de Toulouse, UPS, INPT, CNRS-LAPLACE, 118 Route de Narbonne, 31062 Toulouse, Cedex 9, France
[c] OR-EL.doo, Volariceva Ulica 6, 5222 Kobarid, Slovenija

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ABSTRACT

Integrated biochips are the ideal solution for producing portable diagnostic systems that uncouple diagnosis from centralized laboratories. These portable devices exploit a multi-disciplinary approach, are cost effective and have several advantages including broader accessibility, high sensitivity, quick test results and ease of use. The application of such a device in food safety is considered in this paper. Fluorescence detection of a specific biological probe excited by an optical source is one of the most commonly used methods for quantitative analysis on biochips. In this study, we designed and characterized a miniaturized, highly-sensitive DNA biochip based on a deep-blue organic light-emitting diode. The molecular design of the diode was optimized to excite a fluorophore-conjugated DNA probe and tested using real meat samples to obtain a high sensitivity and specificity against one of the most common poultry meat contaminants: *Campylobacter* spp. Real samples were analyzed also by classical plate methods and molecular methods to validate the results obtained by the new DNA-biochip. The high sensitivity obtained by the OLED based biochip (0.37 ng/μl) and the short time required for the results (about 24 h) indicate the usefulness of the system.

1. Introduction

Campylobacteriosis is considered the most frequent foodborne illness in the European Union (EU), and the most common food contaminated by *Campylobacter* is chicken meat. One of the most important goal in food security is the development of accurate and early diagnosis for foodborne diseases. The European Food Safety Authority (EFSA) estimated that approximately nine million cases of campylobacteriosis occur each year in Europe, with a cost to public health systems of approximately EUR 2.4 billions.

Both classical culture media based and molecular biology techniques used for a long time have limitations: the long time required by the classical methods to grow microorganisms, that can lead to the distribution of contaminated food, and the sensitivity to contaminants of DNA polymerase used in PCR.

Moreover, some bacteria can be stressed by food industry heat treatments and are not able to grow on selective agar plates, (viable but-not culturable, VBNC), thus making plate count methods unsuitable for testing. Molecular biology has greatly improved the techniques by reducing the time required to obtain results. Although real-time-PCR (Levi et al., 2003) allows results to be obtained in a few hours, the inhibition caused by contaminants in the DNA polymerase used in PCR, can produce false-negative results. Moreover, PCR tests are normally run in a laboratory context, while the opportunity to perform point-of-care food controls can improve the safety of food distribution. Recent advances in biosensor technology promise sensitive and specific point-of-care tests with rapid results.

"Different detection technologies have been used in the development of biosensors that can be used for the rapid screening of foods to detect foodborne pathogens prior to distribution, like for example optical sensors (Passaro et al., 2012), acoustic sensors (Jia et al., 2012), microwire sensors (Lu and Jun, 2012) and electrochemical biosensors (Marks et al., 2007)".

Antibodies, cells and DNA have been used as probes in the fabrication of biosensors (Lei et al., 2006). In particular, DNA is a biological element that is useful for the creation of genosensors (DNA-biosensors) (Cecchini et al., 2012), which allow the rapid monitoring of hybridization with the target DNAs. These biosensors, which are based on the oligonucleotide sequences chosen as probes, are specific and sensitive. To reveal the presence of a hybrid generated by the annealing of the DNA probe to the DNA target in various samples, it is

* Corresponding author. Fax: +39 0432558130.
E-mail address: marisa.manzano@uniud.it (M. Manzano).

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possible to use a detected a fluorescent signal. In particular, DNA probes can be labelled with fluorophores, and their weak optical signal can be detected using a charge-coupled device (CCD) camera after a suitable optical excitation.

Various authors (Yao et al., 2005; Hofmann et al., 2005; Pais et al., 2008; Ramuz et al., 2009; Lamprecht, 2010) demonstrated the utilization of an organic light emitting device (OLED) source as a fluorescence excitation source to produce a sensitive biochip.

In particular, the use of an optimized OLED source for the detection of protein arrays has been demonstrated by Marcello et al. (2013). In this work we apply, this OLED light source to a DNA-biochip for the detection of *Campylobacter,* one of the most important pathogens responsible for human gastroenteritis. Campylobacteriosis still causes large economic losses worldwide. Classical methods for *Campylobacter* identification in food samples rely on broth enrichment and colony growth on selective agar plate which takes, at least five days (ISO 10272-1B: 2006) (Voedsel en Warent Autoriteit, 2010). In this work, we tested the sensitivity of a new bio-sensor using both pure culture reference strains and real poultry meat samples to determine the sensitivity of the biochip. The tests results are compared with the standardised laboratory methods including PCR, broth enrichment and colony growth, to demonstrate the relevance of this system for a rapid, simple and reliable point-of-care test for poultry meat.

2. Materials and methods

2.1. OLED fabrication

For this experiment high quality polished borosilicate glass substrates of 1 mm thick coated with 150 nm of indium tin oxide (ITO) of about 20 Ω/square surface resistance have been used. The ITO has been partially removed by a lithographic process using UV curable resins and a mask aligner in a class-10 clean room. Before coating the samples with poly(3,4-ethylenedioxythiophene)-poly (styrenesulfonate) (PEDOT-PSS), an ultrasonic cleaning with organic solvents (acetone, iso-propanol and ethyl alcohol) and demineralized water has been performed on each substrate. All samples have then been dried with nitrogen. After cleaning, the PEDOT-PSS was deposited on the ITO glass substrate at a speed of 2000 RPM for 20 s in the clean room. After the PEDOT-PSS coating, the samples were annealed at 100 °C in air atmosphere for 5 min. After this treatment the samples were transferred into a BOC EDWARDS 500 evaporator, integrated in a pure nitrogen filled JACOMEX glove box, for both the organic layers and the metal cathode deposition. For the organic layers deposition Knudsen cells, each integrated with a thermocouple, for a PID (proportional integral derivative control) setting and a constant monitoring of the cell temperature, have been used. The deposition rate of the organic layers was set at 0.1 nm/s and the film thickness was monitored, during the evaporation, by a calibrated quartz microbalance. A thin LiF layer (1 nm) and a pure aluminium (99.99%) layer was then deposited by electron beam technique in the same BOC EDWARDS 500 evaporator. Two different evaporation rates of 0.01 nm/s and 0.2 nm/s were used for the thin films evaporation, and the film thickness has been monitored by a calibrated quartz microbalance. During both the organic and metal evaporations the pressure in the vacuum chamber was maintained at 1×10^{-6} mbar. After the evaporation the organic light emitting diode (OLED) samples have been encapsulated with a glass lid and a UV curable resin in the glove box. The optical and electrical characterization of the OLED samples was performed in air. The spectral emission and the radiance of the OLED device, measured at normal incidence, was recorded with a GL Spectis 5.0 spectroradiometer (GL Optics GmbH), while the *J–V* curves of the OLED device were recorded with a source metre specifically developed ain the LAPLACE laboratory (Toulouse, France).

Table 1
Reference microorganisms used to test sensitivity and specificity of the DNA probes.

No.	Microorganism	Source
1	*Weissella cibaria*	DSM 14295[a]
2	*Vibrio spp.*	DSM 14379[a]
3	*Escherichia coli*	DISTAM[b]
4	*Pseudomonas aeruginosa*	DISTAM[b]
5	*P. migulae*	DISTAM[b]
6	*P. fluorescens*	DISTAM[b]
7	*P. brennerii*	DISTAM[b]
8	*Bacillus coagulans*	DSM 2308[a]
9	*B. subtilis*	DSM 1029[a]
10	*B. cereus*	DSM 2301[a]
11	*Proteus vulgaris*	DISTAM[b]
12	*Yersinia enterocolitica*	DISTAM[b]
13	*Morganella morganii*	DISTAM[b]
14	*Salmonella Enteritidis*	DSM 4883[a]
15	*Listeria monocytogenes*	ATCC 7644[c]
16	*Citrobacter freundii*	DSM 15979[a]
17	*Enterobacter cloacae*	DSM 30054[a]
18	*Aeromonas sobria*	DSM 19176[a]
19	*Lactobacillus plantarum*	DSM 20174[a]
20	*Pediococcus pentosaceus*	DSM 20336[a]
21	*Leuconostoc lactis*	CECT 4173[d]
22	*Saccharomyces cerevisiae*	ATCC 36024[c]
23	*Campylobacter jejuni subsp. jejuni*	DSM 4688[a]
24	*C. jejuni*	ATCC BAA-1153[c]
25	*C. jejuni*	ATCC 49943[c]
26	*C. coli*	DSM 24155[a]
27	*C. coli*	DSM 24128[a]
28	*C. coli*	ATCC 43478[c]
29	*C. lari subsp. lari*	DSM 11375[a]
30	*C. upsaliensis*	DSM 5365[a]
31	*Helicobacter pylorii* p1	Hospital of Udine[e]
32	*Helicobacter pylorii* p2	Hospital of Udine[e]

[a] DSM: Deutsche Sammlung von Mikroorganism und Zellkulturen GmbH (Braunschweigh, Germany).
[b] DISTAM: Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (Milan, Italy).
[c] ATCC: American Type Culture Collection (Manassas, VA, USA).
[d] CECT: Colección Española de Cultivos Tipo (University of Valencia, Spain).
[e] Isolated from hospitalized patient (Hospital of Udine, Italy).

2.2. Strains selection and DNA preparation

As a first step for the bio-chip construction, 32 microorganisms (31 bacteria and 1 yeast from international collections) listed in Table 1 were used for testing the specificity and sensitivity.

To evaluate the specificity of the designed probes the standardization of the DNA extracted from the different bacteria listed in Table 1 is necessary.

The DNA of the reference strains was extracted and purified from one millilitre of overnight broth culture using the Wizard® Genomic DNA Purification Kit (Promega, Milan, Italy) (Cecchini et al., 2012). The purity and concentration of the DNA samples were evaluated by spotting 1 µl of the extracted DNA onto the spectrophotometer nanodrop 2000c (Thermo Fisher Scientific, Wilmington, DE, U.S.A.) which allow the measure of the DNA avoiding dilution steps. After the reading of the concentrations the samples were standardized at the same concentration using ddwater.

2.3. DNA probe construction and test

A new 55-base DNA detection probe (CampyDet) (5′ CACTTTTCGGAGCGTAAACTCCTTTTCTTAGGGAAGAATTCTGACGGTA CCTAAG-3′) specific for the 16 S rRNA gene of *Campylobacter* spp.