Biosensors and Bioelectronics 87 (2017) 453–458

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

# Amperometric biosensor based on a single antibody of dual function for rapid detection of *Streptococcus agalactiae*

Gersson Vásquez [a], Alba Rey [a], Camilo Rivera [a], Carlos Iregui [a], Jahir Orozco [b,*]

[a] Grupo de Patología Veterinaria, Universidad Nacional de Colombia, Carrera 30 No. 45-03, Bogotá 111321, Colombia
[b] Max Planck Tandem Group in Nanobioengineering, Universidad de Antioquia, Complejo Ruta N, Calle 67 No. 52-20, Medellín 050010, Colombia

ARTICLE INFO

*Article history:*
Received 4 July 2016
Received in revised form
15 August 2016
Accepted 25 August 2016
Available online 26 August 2016

*Keywords:*
*S. agalactiae*
Screen-printed carbon electrode
Immunosensor
Biotinylated antibody
Amperometric detection

ABSTRACT

Pathogenic bacteria are responsible for several diseases in humans and in a variety of hosts. Detection of pathogenic bacteria is imperative to avoid and/or fight their potential harmful effects. This work reports on the first amperometric biosensor for the rapid detection of *Streptococcus agalactiae* (*S. agalactiae*). The biosensor relies on a single biotinylated antibody that immobilizes the bacteria on a screen-printed carbon electrode while is further linked to a streptavidin-conjugated HRP reporter. The biotinylated antibody provides selectivity to the biosensor whereas serves as an anchoring point to the reporter for further amplification of the electrochemical signal. The resultant immunosensor is simple, responds rapidly, and allows for the selective and highly sensitive quantification of *S. agalactiae* cells in a concentration range of $10^1$–$10^7$ CFU ml$^{-1}$, with a detection limit of 10 CFU ml$^{-1}$. The approach not only enables a rapid detection and quantification of *S. agalactiae* in environmental samples but also opens up new opportunities for the simple fabrication of electrochemical immunosensors for different target pathogens.

© 2016 Elsevier B.V. All rights reserved.

## 1. Introduction

*S. agalactiae* is a Gram-positive pathogen that affects mainly human, cattle and fish whereas can cause sporadic diseases in many other hosts (Elliott et al., 1990; Hetzel et al., 2003; Johri et al., 2006; Yildirim et al., 2002). This bacterium is responsible for several pathologies, such as neonatal meningitis and sepsis in human beings, mastitis in cattle and meningoencephalitis, epicarditis, and choroiditis in fish (Evans et al., 2002; Hernández et al., 2009; Mian et al., 2009; Mitchell, 2003; Oliveira et al., 2005). However, the phylogenetic relationships among the *S. agalactiae* populations in the three hosts have not been clearly established (Delannoy et al., 2016; Finch and Martin, 1984; Pattison et al., 1955). *S. agalactiae* has shown to have a profound impact in both, public health and aquaculture. For example, as a primary commensal bacterium of the gastrointestinal and genitourinary tracts, *S. agalactiae* is commonly misdiagnosed because it coexists with multiple bacteria populations, affecting public healthcare systems especially in developing countries (Timoney, 2010). It is the major cause of morbidity and mortality in Tilapia, producing international economic losses up to $ 150 million for the aquacultural industry (Amal and Zamri-Saad, 2011). A precise identification of the bacterium and the source of its derived infections and determination of dissemination pathways and its maintenance in the different hosts and environments are prevailing questions that are currently under investigation. Therefore, the development of a fast, sensitive, and accurate analytical tool for detection of *S. agalactiae* in patients, in animals and in their natural habitats is of high priority.

Although significant efforts have been devoted to the development of diagnosis tools for pathogens, most technologies are still far from ideal, being either time consuming or complex (requiring specialized personnel and equipment); or unspecific and not sensitive, costly and not available in all laboratories (Fournier et al., 2013). Current ways for identifying *S. agalactiae* are based on bacteriological examinations (Keefe, 1997), serological methods (Skinner and Quesnel, 1978) and PCR, while histopathology is frequently implemented in fish pathology (Iregui et al., 2014). Diagnostic tests and devices based on biosensors are being increasingly tested as alternatives to standard laboratory instrumentation for clinical diagnosis (Elliott et al., 1990; Liébana et al., 2009; Sotillo et al., 2014) and environmental monitoring (Orozco and Medlin, 2011; Orozco et al., 2009). Among them, the high affinity of those based on antibodies has been extensively demonstrated (*e.g.* Mendoza et al., 2008). Biosensors are highly sensitive and respond rapidly. They are inexpensive, easy to operate and can be integrated into portable and automatic measurement systems. Only one biosensor for detection of *S. agalactiae*

* Corresponding author.
*E-mail address:* grupotandem@udea.edu.co (J. Orozco).

http://dx.doi.org/10.1016/j.bios.2016.08.082
0956-5663/© 2016 Elsevier B.V. All rights reserved.

in clinical samples has been published so far, using impedance spectroscopy as the detection system (Chiriacò et al., 2016). However, to the best of our knowledge, this work is the first amperometric biosensor for screening the bacterium in environmental samples.

Amplification is a crucial step in the fabrication of biosensors. Different strategies for amplification of the analytical signal have been explored, including incorporation of nanomaterials, biomaterials or a combination of them into nanobiocomposites (Crespilho et al., 2009). Amplification of biotinylated sites can intensify histochemical reactions to increase the sensitivity of staining procedures up to 100-fold employing horseradish peroxidase (HRP) and/or biotin in tissues (Adams et al., 1996). Such histopathologic affinity reactions have inspired the development of the biosensor format presented here. Biotin can be linked to different molecules, and then attached with high affinity and specificity to avidin (neutravidin or streptavidin), which, in turn, can be coupled to enzymes, nanoparticles, or fluorochromes etc., for a versatile range of application in (bio)sensing (Bobrow et al., 1989).

Herein, we describe the development of an amperometric immunosensor for the detection of *S. agalactiae* isolated from Tilapia fish. The biosensor relies on a single biotinylated antibody of dual function that selectively immobilizes the bacterium on a screen-printed carbon electrode (SPCE) surface while is further linked to a streptavidin-conjugated HRP reporter for amplification of the electrochemical signal. The resultant immunosensor is fast, simple, selective and highly sensitive. The protocol led to an efficient signal amplification with reduced assay steps and incubation times. The approach permitted the rapid screening of *S. agalactiae* in environmental samples of interest to the Tilapia fish industry. However, it could be implemented in human *S. agalactiae* clinical monitoring, opening up new opportunities for the fabrication of simple electrochemical immunosensors for different target pathogens.

## 2. Materials and methods

### 2.1. Equipment and reagents

Electrochemical measurements were performed using a potentiostat-galvanostat PalmSens and SPCEs with a standard three-electrode configuration: platinum, silver, and carbon as the counter, reference and working electrodes, respectively, DRP-150 (Dropsens, Oviedo-Spain). NeutrAvidin (Molecular Probes); biotinylated polyclonal anti-*Streptococcus* group B antibody (ab 19983) and 3,3′,5,5′-Tetramethylbenzidine (TMB) containing hydrogen peroxide (abcam); Streptavidin Horseradish Peroxidase (HRP) SA-5004 (Vector Laboratories); were used as received. Phosphate buffered saline (PBS) 1X pH 7.2 and 7.4; PBS pH 7.2, containing 0,05% Tween-20; 0,05 M $NaHCO_3$ buffer pH 9.6 and 0,01 M acetate buffer pH 5.0, were prepared with deionized sterilized water.

### 2.2. Bacteria cultivation

*S. agalactiae* reference strain (SaTiBe0818) was cultivated in brain heart infusion (BHI) agar plates for 24 h at 37 °C and the cultures resuspended in sterile PBS. Optical density (OD) of bacterial culture was measured to determine the bacterial growth stationary phase. Cultures of *S. agalactiae* strain were grown to late log phase (OD600 = 0.4). The number of viable cells was determined by the spread-plate technique.

### 2.3. Fabrication of the immunosensor

The SPCEs were incubated overnight in 7 μl of 100 μg/ml neutravidin dissolved in 0,05 M $NaHCO_3$ solution, at 4 °C covering all electrodes when placed in a wet chamber. After neutravidin adsorption, the electrodes were rinsed with PBS-tween buffer (3 times) and 3% bovine serum albumin (BSA) was dropped onto the chips for 1 h for blocking of unspecific sites. Bacteria were pre-incubated in a 1–100 μg/ml solution of anti-*S. agalactiae* antibodies, under agitation for 30–90 min. The resultant bio-conjugated was dropped onto the chips and incubated for 15–60 min with further rinsing with PBS-Tween buffer (3 times). Finally, the SPCEs were incubated in a 1–10 μg/ml streptavidin-HRP conjugated for 30 min and washed 3 times with PBS-Tween buffer. The amperometric signal was recorded by placing 45 μl 0,01 M acetate buffer pH 5.0 on the SPCE surface. Current was recorded at −200 mV/s for 60 s, after which 5 μl TMB were added to the electrochemical cell and the current further registered for additional 140 s (Salam and Tothill, 2009). HRP molecules catalyze the enzymatic oxidation of TMB in the presence of $H_2O_2$. The oxidized TMB is reduced back at the surface of the SPCE, thus producing a signal that is proportional to the number of cells. We optimized the biosensor fabrication process in terms of sensitivity, reproducibility and time of analysis. The parameters were sequentially changed at a time while keeping others constant. At optimal conditions, we studied the biosensor response towards different *S. agalactiae* bacterial concentrations and results were processed using the PStrace 4.2 software.

### 2.4. Selectivity and environmental samples testing

Selectivity was evaluated by incubating the immunosensor in $10^5$ CFU of *S. agalactiae* isolated from Tilapia fish suffering from streptococcosis and comparing its response to that from incubation in the same number of CFU of two species of bacteria that commonly coexist with *S. agalactiae* in Tilapia (i.e,. *Aeromonas hydrophila* and *Edwardsiella tarda*), under the same experimental conditions. The response of the immunosensor with all the reagents but in the absence of bacteria and only in buffer were also included as negative controls. Finally, we evaluated the immunosensor response in samples of interest from the fish industry. Current intensities of the immunosensor were tested in samples from different farm water sources named Source 1, Pond 1 and Sludge 1 (4°15′42″N, 73°33′51″W) and Source 2, Pond 2, Sludge 2 (3°41′49″N, 73°41′55″W) from the Colombian departments of Tolima and Meta, respectively. Samples from Lake Betania located at Huila, Colombia (2°41′6″N, 75°26′24″W), in absence of the bacteria or inoculated with $10^1$, $10^4$ and $10^7$ *S. agalactiae* cells were tested as negative and positive controls, respectively.

## 3. Results and discussion

### 3.1. Immunosensor optimization

The underlying immunosensor principle relies on the immobilization of *S. agalactiae* bacteria on the surface of a neutravidin-coated SPCE and further amplification of the amperometric signal with streptavidin-linked HRP enzyme. SPCEs were selected taking into account their outstanding electrochemical properties and having in mind a further application of the resultant biosensor in field settings. The first parameter to be optimized was the antibody concentration and its incubation time. In preliminary experiments, biotinylated antibodies solutions of different concentrations were first anchored on the surface of a neutravidin coated SPCE, as explained above, and the bacteria linked (and further labeled) as described. However, the resultant amperometric signals were very poor independent of the antibody concentrations and incubation times (data not shown).

Download English Version:

https://daneshyari.com/en/article/7230193

Download Persian Version:

https://daneshyari.com/article/7230193

Daneshyari.com