Journal of Microbiological Methods 75 (2008) 545–550

Contents lists available at ScienceDirect

Journal of Microbiological Methods

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

A rapid serological assay for prediction of *Salmonella* infection status in slaughter pigs using surface plasmon resonance

Saikat Datta Mazumdar [a], Benjamin Barlen [a], Thomas Kramer [b], Michael Keusgen [a,*]

[a] *Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Marbacher Weg 6, D-35032 Marburg, Germany*
[b] *Labor Diagnostik GmbH Leipzig, Deutscher Platz 5b, D-04103 Leipzig, Germany*

ARTICLE INFO

Article history:
Received 7 April 2008
Received in revised form 19 August 2008
Accepted 29 August 2008
Available online 5 September 2008

Keywords:
Enzyme-linked immunosorbent assay (ELISA)
Lipopolysaccharide (LPS)
Pig serum
Salmonella Typhimurium
Serological assay
Surface plasmon resonance (SPR)

ABSTRACT

We present a rapid surface plasmon resonance-based serological assay for the detection of *Salmonella* Typhimurium infection in pigs using the Plasmonic® SPR device. Lipopolysaccharide (LPS, 10 μg mL^{-1}) from *Salmonella* Typhimurium was immobilised by self-assembly on a hydrophobic SPR chip. Using this LPS-coated chip, it was possible to bind and detect the anti-*Salmonella* Typhimurium antibodies in serum of pigs infected with the bacteria. The developed SPR assay is able to differentiate between sera obtained from pigs having low, medium, and high levels of *Salmonella* infection. A commercial ELISA kit was used to classify the sera for levels of *Salmonella* infection on the basis of optical density (OD%). A strong positive correlation was observed between the SPR-based assay and the ELISA ($n=38$, $r=0.90$, $p<0.01$). The sensitivity and specificity of the assay are 0.93 and 0.87, respectively. The SPR-based assay is label-free and does not require any sample preparation or dilution steps. The total analysis time is 45 min for each serum sample. The assay was found to be specific for *Salmonella* Typhimurium and shows no cross-reactivity to *Salmonella* Choleraesuis or *Escherichia coli* antibodies. As no sample preparation is required the developed assay has the potential to be used as a reliable tool for *Salmonella* monitoring programmes in pork production.

1. Introduction

The directive 2003/99/EC of the European parliament and the Council of the European Union outlines the strategy for the monitoring of zoonosis and zoonotic agents in Europe (Anonymous, 2003a). Control of *Salmonella* infection in pigs forms an important part of the Regulation (EC) No 2160/2003 of the European Parliament (Anonymous, 2003b). Article 4 of this regulation states that Community targets shall be established for the reduction of the prevalence of *Salmonella* in herds of slaughter pigs. To set the Community targets, a survey has been initiated to monitor the prevalence of *Salmonella* in slaughter house pigs. As part of this survey, slaughterhouse sampling, by taking ileocaecal lymph nodes to reflect the *Salmonella* status of the pigs sent to slaughter, has been recommended. This method is a culture-based technique, which involves a series of time-consuming steps of pre-enrichment, selective-enrichment, isolation and biochemical identification. In addition, serological methods have also been recommended for use within this programme (Anonymous, 2006).

Timely detection and control of *Salmonella* spp. in pigs is important for the production of *Salmonella*-free pork (Berends et al., 1997; Pearce et al., 2004; Swanenburg et al., 2001; Vieira-Pinto et al., 2006). Serological monitoring of *Salmonella* infection in pigs forms the basis of all successful *Salmonella* monitoring programmes. The most successful and well-known is the Danish *Salmonella* surveillance programme, established in 1995 (Mousing et al., 1997). This programme is primarily based on antibody analysis of meat juice samples using the so-called "mix-ELISA" (Nielsen et al., 1998). The programme relies on these serological results to assign herds to one of the three levels of infection – no or few antibody positive-finisher pigs (level 1), moderate antibody positive-finisher pigs (level 2), and high proportion of antibody positive-finisher pigs (level 3) – on a monthly basis (Sorensen et al., 2004). The herd is considered positive if at least one animal is found infected (Steinbach et al., 2002). Furthermore, the success or failure of *Salmonella* control programmes has commercial implications to the pork trade (Bogetoft and Olesen, 2004; Reimer and Carstensen, 2006). Hence, rapid and reliable detection techniques for monitoring of *Salmonella* infection in pigs need to be explored.

Surface plasmon resonance (SPR) biosensors belong to the category of evanescent wave biosensors. When a light beam passes from a material having a relatively high refractive index (e.g., a glass prism) into a material having a lower refractive index (e.g., water), the light is bent towards the plane of interface. Total internal reflection (TIR) occurs when the angle, at which the light strikes the interface, is greater than the critical angle. SPR is observed under conditions of TIR when the surface of the prism is coated with so-called "free electron metals" such as gold. Surface plasmons are present on the gold surface due to the free-oscillating electrons. At a particular angle of incidence,

* Corresponding author. Tel.: +49 6421 28 25809; fax: +49 6421 28 26652.
E-mail address: keusgen@staff.uni-marburg.de (M. Keusgen).

0167-7012/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.mimet.2008.08.011

the wavelength of the incident light matches with the wavelength of the surface plasmons, resulting in SPR. This angle, at which there is a minimum in the intensity of reflected light, is called the SPR angle.

When SPR occurs, there is also an evanescent electrical field (EEF) generated. This EEF travels from the metal into the sample medium that is in contact with the gold surface. As a result of this phenomenon, the amplitude of the surface plasmon waves changes with the change in refractive index of the sample medium. Consequently, the angle at which SPR occurs also changes. Biomolecular interactions on the surface of the SPR chip cause changes in the refractive index of the sample medium. This is recorded as a shift in the SPR angle with time, resulting in SPR sensograms (Ekgasit et al., 2004). For each SPR angle there is a minimum in the intensity of reflected light. This change in angle, corresponding to the minimum in reflected light, is directly proportional to the loading of biomolecules on the gold surface of the SPR prism (Keusgen, 2002).

SPR has been evaluated for the detection of *Salmonella Enteritidis* antibodies in eggs (Thomas et al., 2006). The use of SPR has been reported for the detection of *Mycoplasma hyopneumoniae* antibodies in swine serum using a protein-based SPR chip (Kim et al., 2006). The detection of antibodies in pigs against classical swine fever virus using a similar SPR assay has also been recently reported (Cho and Park, 2006). *Salmonella* Typhimurium is the serotype most often isolated in pig farms with clinical Salmonellosis (Coma, 2003). However, to our knowledge, there is no literature available on the detection of *Salmonella* Typhimurium antibodies in infected pigs using SPR. Given the importance and prevalence of *Salmonella* Typhimurium in pigs and the advantages of the SPR technique, being rapid and label-free (Lundstrom, 1994), development of an SPR-based assay for the detection of *Salmonella* Typhimurium in pigs could be of significance to the pork industry.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide (LPS) from *Salmonella* Typhimurium, prepared by the phenol extraction method, was obtained from Sigma-Aldrich (Germany). O-specific (O:5) antibody against *Salmonella* Typhimurium was obtained from SIFIN (Berlin, Germany). Sera from both healthy and infected pigs were obtained from pig farms across Germany and were provided by Labor Diagnostik GmbH Leipzig (LDL, Germany). For the purpose of cross-reactivity tests, sera from pigs infected with *Salmonella* Choleraesuis were used. Antibodies against *Escherichia coli* used in the cross-reactivity tests were obtained from Fitzgerald (Concord MA, USA). The SALMOTYPE® Pig Screen ELISA kit, used as a comparison, was also obtained from LDL. Bovine serum albumin (BSA) was purchased from Merck (Darmstadt, Germany). Phosphate buffered saline (PBS, 0.15 M, pH 7.3, containing 0.12% Bronidox® L) used in all experiments was prepared in our laboratory. Water used was obtained from a PURELAB® Plus unit (USF Elga, Germany). All other chemicals were purchased from standard commercial sources and were of analytical grade.

2.2. Sample preparation

The sera from *Salmonella*-infected and -uninfected pigs (negative control serum) were received from LDL, either frozen or in freeze-dried form. Frozen samples were thawed prior to use. Freeze-dried samples were reconstituted in water to their original volume prior to use. A total number of 38 serum samples obtained directly from pig farms were screened for *S.* Typhimurium infection. For the SPR assay, required dilutions of sera were either carried out using PBS or negative control serum. In case of ELISA, sera were diluted 1:100 using the dilution buffer supplied with the SALMOTYPE® Pig Screen ELISA kit. The LPS from *Salmonella* Typhimurium, immobilised on the SPR chip, was prepared by dissolving the freeze-dried LPS in PBS.

2.3. Surface coating

The gold surface of each prism was modified to create a hydrophobic surface, henceforth referred to as C18. This method of modification of the SPR gold surface has been recently reported by our working group (Mazumdar et al., 2007, Barlen et al., 2007). Briefly, the gold prisms were first cleaned in acetone for 5–10 min, followed by incubation in a mixture of 0.1 M potassium hydroxide and 30% hydrogen peroxide for 20 min. The gold prisms were then rinsed with water, followed by incubation in a solution of C18-alkylsilane for 6 h at room temperature. The prisms were then dried under vacuum. The C18 gold prisms were then stored under vacuum until further use.

2.4. Surface plasmon resonance device

The assay described here was developed on the Plasmonic® SPR device (Plasmonic Biosensoren AG, Wallenfels, Germany). The device (Fig. 1) is characterised by a cuvette system, having eight channels, which are filled by means of an autosampler, eliminating any microfluidics. The gold surface of the SPR prisms forms the bottom

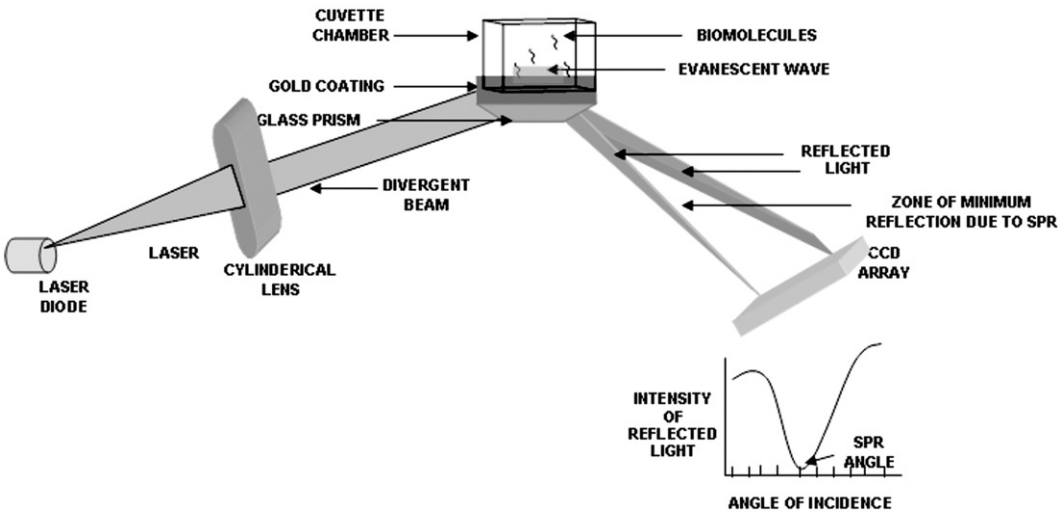

Fig. 1. Schematic representation of the detection of biomolecular interactions using the Plasmonic® SPR device, showing the defocussing optics (laser diode, 786 nm) and the cuvette chamber placed on the gold-coated (50 nm) prism. The chamber is further subdivided into eight small channels, enabling measurements of eight different samples on one single chip.