Surface Plasmon Resonance assay for monitoring of coliphages in waste water

Introduction

Surface Plasmon Resonance is a surface sensitive optical technique that can be used to detect biological interactions from protein – antibody binding to nucleic acid hybridization. This method was also successfully used to determine viral infections by detecting antibodies directed to virusses in serum samples. This application note describes a method to show the presence of live coliphages in waste water samples.

Coliphages

Coliphages are virus-like particles that infect *Escherichia coli* bacteria (*E. coli*) for their proliferation. Their presence in surface water indicates the contamination of this water with waste water, as their source is the faeces from humans and animals. The coliphage count, therefore, is used as an indicator of waste water contamination.

The conventional way to determine the coliphage count is the double-layer agar assay: A lawn of *E. coli* bacteria, grown in a nutrient agar, is infected with a phage containing water sample.

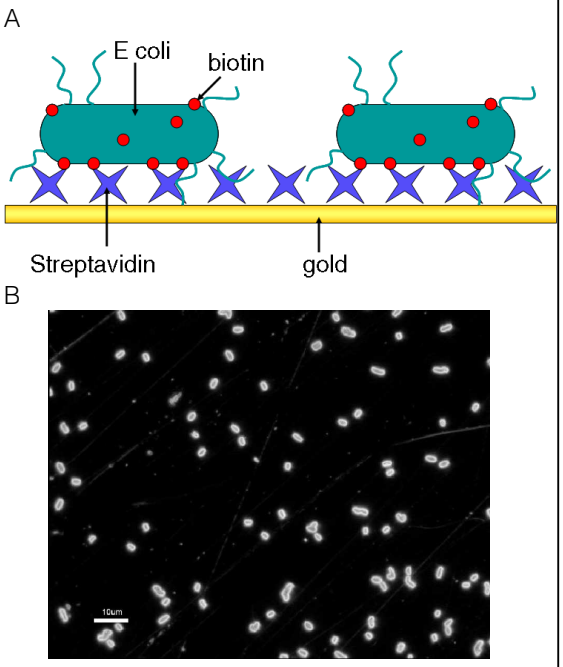

Figure 1: Bacteria immobilized on gold disk A: schematic presentation B: Microscopic view of the E. coli immobilized on the gold surface.

Upon infection, the bacteria undergo lysis, forming a lesion in the lawn, a plaque. The number of plaques determines the amount of plaque forming units (PFU) in the sample. The SPR technique offers an alternative, fast way to determine the presence of coliphages in waste water samples in a real time measurement.

Method

E. coli bacteria were biotinilated by incubation in 1 mg/ml sulfo-NHS-XX biotin in ice cold PBS. Streptavidin was directly adsorbed to the gold disk. In the cuvette of the SPR instrument, the biotin-tagged bacteria were allowed to bind to the streptavidin in PBS buffer. This resulted in irreversible attachment of bacteria on the gold surface. Figure 1 shows a schematic and a microscopical presentation of the bacteria bound to the gold disk.

Measurement

On this bacterial film, the SPR response to samples containing coliphages was measured. Samples with different concentrations of coliphages were applied to the bacterial layer and the response was followed during the incubation without mixing. The SPR response is shown in figure 2. The initial increase in SPR angle is caused by the binding of the bacteria. The increase in the SPR signal following the PBS wash is attributed to change in the structure of the attached bacterial film. The ESPRIT SPR instrument allows a reference measurement in the second channel.

Some time after the injection of the coliphage sample, a sudden increment of the SPR angle

is seen only in the experimental channel. The time delay of the increase in the angle is inversely proportional to the concentration of bacteriophages in the sample (figure 3A and 3B). As few as 100 PFU/ml could be detected after an incubation time of 120 min.

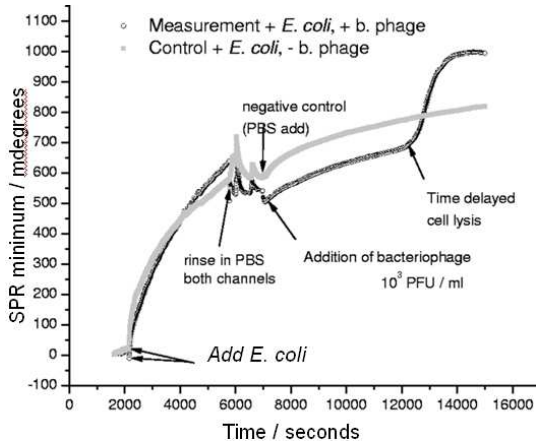

Figure 2. Typical SPR measurement of coliphage presence in waste water.

The shift in angle is hypothesized to be caused by newly released phage particles, bacterial protein content and bacterial membrane fragments, released after the collapse of the bacteria, that bind to the gold surface. A similar angle shift was reported after cell lysis induced by the membrane lysing enzyme phospholipase A2 (results not shown).

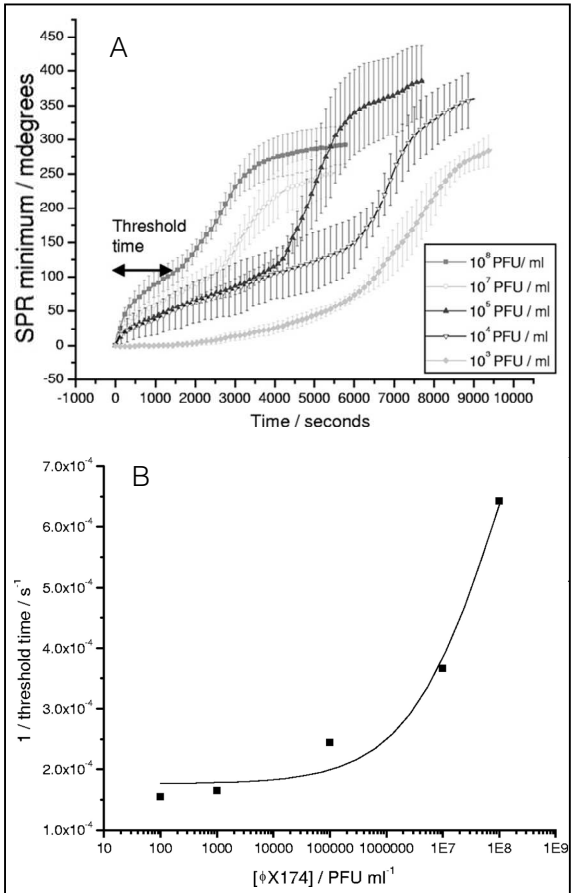

Figure 3 A: Response curves of different concentrations of coliphages. B: Response times versus coliphage concentration.

Several samples from waste water treatment plants were evaluated for the presence of coliphages by both double-layer agar method and SPR. The time lapse before the increase in signal corresponded with the coliphage concentration detemined by the double-layer agar method.

Conclusion:

This new approach of real time monitoring with the Autolab ESPRIT for the presence of coliphages can be applied to samples which need to be screened in a short period of time. This SPR approach of biosensing is highly specific and highly sensitive.

These results show the suitability of the Autolab SPR instruments for the development of biosensors for the detection of bacteriophages and other faecal indicators in waste water.

This application note is an adaptation of the paper by:

Cristina García-Aljaro, Xavier Muñoz-Berbel, Toby A. Jenkins, Anicet R. Blanch and Francesc Xavier Muñoz.
Surface Plasmon Resonance Assay for Real-Time Monitoring of Somatic Coliphages in Wastewaters. 2008, *Applied and Environmental Microbiology* 74; 4054–4058