Lytic phage in biosensing
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Methicillin-resistant Staphylococcus aureus (MRSA) is a bacterium accountable for a number of difficult-to-treat diseases in humans. Recently, cases of MRSA have increased in livestock animals from which the infection can be transmitted to humans. The rapid and reliable techniques for recognition of MRSA are needed.

Lytic phage biosensors and antibody beads are capable to discriminate between methicillin resistant (MRSA) and sensitive staphylococcus bacteria. The phages were immobilized by a Langmuir-Blodgett method onto a surface of a quartz crystal microbalance sensor2 and worked as broad range staphylococcus probes. Antibody beads recognize MRSA.

The overall goal of the research is to discriminate methicillin resistant (MRSA) and sensitive (MSSA) strains of Staphylococcus aureus, by the specially selected strains and a penicillin-binding protein (PBP 2a) specific antibody, using a quartz crystal microbalance.

This is achieved by recognizing the main obstacle of using an intact lytic phage as a detector probe with quartz crystal microbalance. In the case of intact phage, when bacteria are attached to the long flexible tails, the distance between attached cells and a sensor surface is larger than penetration depth of QCM’s acoustic wave. The acoustic wave cannot “reach” bacteria and therefore the signal of penetration between attached cells and a sensor surface is larger than bacteria are attached to the long flexible tails, the distance of the QCM’s acoustic wave. The acoustic wave cannot “reach” bacteria and therefore the signal of the binding event is not generated. This phenomenon is known as a “missing mass” problem in biosensor’s QCM applications. (Fig. 1)

The problem of “missing mass” in this work is easily solved by replacing the intact phage with spheroids, the phage modified by chloroform-water treatment. This treatment results in fully functional phage with a short tail. The bacterium bound to the tail becomes positioned within the penetration depth of QCM’s acoustic wave, came into “traction” with the oscillated resonator and therefore contributes to the frequency and dissipation change (produces a signal). (Fig. 2)

When phage spheroids (phages with short tails) are deposited on a QCM-D crystal and exposed to the mixed bacterial suspension, they bind both methicillin resistant (MRSA) and sensitive (MSSA) strains of Staphylococcus aureus, but do not bind other bacteria. In this important step, S. aureus are separated from the other bacteria on the crystal surface. (Fig. 3).

Finally, to discriminate MRSA and MSSA, the suspension of beads with PBP 2a specific antibody are added. The beads bind only MRSA, that contain PBP 2a protein and do not bind MSSA, which missing this protein. When beads bind MRSA, the signal is generated indicating the presence of methicillin resistant (MRSA) strains of Staphylococcus aureus (Fig. 4).

The created biosensors have been examined by a 4-chamber quartz crystal microbalance with dissipation tracking (QCM-D) to evaluate bacteria-phage interactions. Bacteria-spheroid interactions led to reduced resonance frequency and a rise in dissipation energy for both MRSA and MSSA strains. After the bacterial binding, these sensors have been further exposed to the penicillin-binding protein antibody latex beads. Sensors analyzed with MRSA responded to PBP 2a antibody beads; although sensors inspected with MSSA gave no response. This experimental distinction determines an unambiguous discrimination between methicillin resistant and sensitive S. aureus strains.

A total time-to-answer for this assay is about 16 minutes per sample. This time can be dramatically shortened by using QCM devices with a large number of chambers. The anticipated shelf life for the phage sensors is about of 3-4 months at room temperature. With a biopolymer protection it could be prolong up to a few years.2 The detection limit of S. aureus that was measured for this phage was found to be 105 CFU/ml.

Commonly used methods for detection of MRSA require from 3 to 24 hours carrying out the test. PCR or DNA hybridization of the mecA gene is a relatively fast and accurate method but requires purified DNA and is extremely sensitive impurities. In contrast, the method described in this work is rapid, does not need DNA extraction, and it is not sensitive to admixtures.

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REFERENCES