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IMMUNE BIOSENSORS FOR DIAGNOSTICS AND CONTROL OF *SALMONELLA TYPHIMURIUM*

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Several types of biosensors based on optical (SPR, TIRE), potentiometric (ISFET) transducers and one based on ZnOnanorods are proposed for S. typhimurium detection. An effect of preliminary preparation of transducers to the sensitivity of analysis was analyzed. It was found that all proposed biosensors were effective for S. typhimurium detection in model solutions and can be used in practice at different levels of diagnostics. The highest sensitivity was obtained using TIRE based biosensor that was several cells (less than 5) in 10 ml.

Keywords: *Immune biosensor, antigen-antibody reaction, ZnOnanorods, Salmonella typhimurium.*

Salmonella is one of the most common causes of foodborne disease worldwide. In epidemiological meaning, only some bacteria of *Salmonella* take an important place and cause 85-91% cases of salmonellosis and *S. typhimurium* is among them [1]. For human thesource of poisoning are meat, eggs, fish, dairy products, fresh fruits and vegetables contaminated by living microorganisms [5]. Infectious dose is slightly different depending on the authors. The most likely it is due to the difference of state standards for countries. Some authors indicate the infectious dose for *Salmonella* at the level of 10 cells and its presence in the water at the level of 4 cells in 100 ml [7]. According to Mandate of the Ministry of Health of Ukraine №400 12.05.2010. On approval of the State sanitary rules and regulations "Hygienic requirements to drinking water intended for human consumption" microbiological

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requirements for drinking water provide absence of pathogenic enterobacteria in 1 liter of water [2].

To protect the population from pathogens it is necessary to conduct continuous monitoring of the environment. Unfortunately, traditional methods of *S. typhimurium* determination does not fully meet the practical demands, as they are difficult and include pre-enrichment, selective enrichment, biochemical testing and serological confirmation. Culture method of *Salmonella spp*. detection requires 3 – 4 days for further biochemical confirmation [9].

As alternative methods of *Salmonella* detection,enzyme-linked immunosorbent assay (ELISA) and different kinds of polymerase chain reaction (PCR) are widely used. Nevertheless, these methods also have several disadvantages such as low sensitivity, cross reactivity, the loss of the distinctive ability of antibodies, requirement of special equipment and long duration of analysis [3]. In this regard, development of cheap and fast methods for pathogens detection in the environment is very topical issue.

The purpose of the work is to develop fast and highly sensitive methods of express diagnostics of *S. typhimurium*.

Materials and methods. Different types of sensor systems had been used in the work, such as optical, potentiometric and ZnOnanorods. Polyclonal antibodies (Ab), specific to *S. typhimurium* (Ag) had been used as selective biological element, and antigen-antibody reaction was the basis of biosensors operation. In order to increase sensors sensitivity transducerpreliminary preparation took place. *S. typhimurium* cells were detected in model solutions, which included suspension of autoclaved cells of *S. typhimurium*in saline solution with initial concentration of 10^9 cells/ml which was further diluted.

Optical biosensors. Sensors based on the phenomenon of surface plasmon resonance (SPR) such as Spreeta (USA) and sensor “Plasmonotest” (property of V.M. Glushkov Institute of Cybernetics of National Academy of Sciences, Ukraine; pat. UA 100934) and sensor based on total internal reflection ellipsometry (TIRE)were used. Direct method of analysis was used in all cases of *S. typhimurium* detection

using optical immune biosensors. As far as using of bare gold surface is not suitable for the interactions of biological molecules previous modification of the transducer surface took place, which included covering of surface by polyalylamine hydrochloride (PAA) and protein A from *Staphylococcus aureus*. Transducer surface was covered with bovine serum albumin (BSA) for blocking free non-specific binding centers on the gold surface. In case of TIRE based biosensor previous modification of the transducer surface was made the same way as for SPR-sensors.

Potentiometric biosensors. Ion-sensitive field-effect transistors (ISFET) with CeOxinsteand of Si_3N_4 gate surface were used as a transducer since the last were less sensitive to pH changes. ISFETs were activated by aqueous solution of glutaraldehyde (GA) with further analysis algorithm as for SPR-biosensors. Blocking free groups of GA was provided by solution of glycine. It was chosen "sandwich" algorithm of analysis. For this purpose *S. typhimurium*cells on the surface treated for 10 min with a solution of specific antibodies, which were labeled by horseradish peroxidase (HP).

Biosensors based on ZnOnanorods. Principle of *S. typhimurium* detection was based on phenomenon of ZnOnanorods photoluminescence (FL) at room temperature. Structures of ZnOnanorodswere obtained by gaseous-disperse synthesis [8]. The method of ZnOnanorods powder deposition and their main characteristics are described in [4]. To test biosensor response the PL spectra were recorded from pure ZnO, from ZnO with a layer of immobilized Anti-*Salmonella* antibodies, from ZnO with BSA blocking agent, and finally after exposure of ZnO-Anti-S*almonella* Ab layer to the target *Salmonella* antigens. After the probe testing experiments, the sensitivity of the prepared biosensor was studied in a wide range of *Salmonella* Ag concentrations from 10^1 to 10^6 cell/ml. As BSA was used as a blocking agent to prevent non-specific interactions, the PL intensity after BSA immobilization was used as an initial point for calculation of the sensor response [4].

Results and discussion. In the case of *S. typhimurium* detection using Spreeta based biosensor, it has been defined that device sensitivity was on the level $10^3 – 10^7$ cells/ml. "Plasmonotest" detection level was within $10^1–10^6$ cells/ml. Biosensor

based on the TIRE has shown higher sensitivity than the SPR based. Maximal level of sensitivity was on the level of several cells (less than 5) in 10 ml.

Biosensor based on the ISFET has shown sensitivity within 2 – $5x10^5$ cells/ml. In addition to high sensitivity this method allows to use biochips several times (up to 5) without signal reduction. For this purpose biochips were treated by 0,1 M HClfor 5 min.

The detection threshold of the ZnOnanorods based biosensor was10^2 cells/ml of Ag concentration. The sensitivity is almostlinear only at the highest values of *Salmonella* antigen concentration (10^2 – 10^5 cell/ml).

It was proved that preliminary preparation of working surface aimed to produce antibody-oriented layer had a significant influence to the level of sensitivity of devices. As a result of physical adsorption of antibodies directly on the gold surface of the biosensor “Plasmonotest” the device sensitivity was 10^4 – 10^6 cells/ml.

Since time of each reagent immobilization to thetransducer surface is on average 10 minutesthe duration of a full cycle of analysis is less than 1 hour. This time can be significantly reduced when previous immobilization of PAA, proteinA and specific antibodiesisprovided, e.g., ELISA provides *S. typhimurium* detection on the level 10^4 cells/ml [10], 10^6 cells/ml [6] with total time of analysis from 6 hours.

Conclusions

Taking into accountthe above resultsone can conclude that all analyzed immune biosensorsare effective for *S. typhimurium* detection in model solutions and are alternative to known todaylaboratory diagnostic methods.TIRE based immune biosensor demonstrated the highestsensitivitythat satisfies practicalneeds.Biosensor based on the ISFET had lower sensitivity but it providesstability and high reproducibility. Regeneration of working surfacereduces the cost of analysis. Other biosensorsshowed lower sensitivity in *S. typhimurium* detection, but still it was higher than sensitivity of ELISA. In addition, they provides rapid analysis in real time,do not require special stafffor analysisand can be usedat certain stages of diagnostics that will make it much faster.

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