Bioreceptor Development for the Detection of *Salmonella* sp.

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One of pathogenic bacterium, which is related to break out of microbial diseases by foods, is *Salmonella*. Recently, because of opening imported food and increasing meal service, food poisoning is frequently occured by food harm microorganism. However, the illness caused by these kind of factors makes us cost massively from the viewpoint of economy and health. So, prevention has been regarded as an important thing. It is important to develop the biosensor which can detect the bacteria so as to prevent infection and proliferlation of the pathogenic bacteria. The biosensor is sensitive to the substrate, reacting promptly, and it is available to analyze with the small amount of the sample. On the other hand, there were lots of problems regarding the sensitivity of reaction, safety of the antibody, availability of reusing and standardizing, productivity and preservation of the antibody. Because of this, when the chip is prepared, it is important to choose the right method which can reduce the nonspecific binding and denaturation of protein. For this, invasion (HilA) and flagellin (FliC) were chosen as the specific proteins for the detection of *Salmonella*, and then those genes were cloned and obtained pure protein through the gene cloning and overexpression system. For the strong epitope prediction of antigenic protein domain, the region where hydrophobicity is low and antigenicity is high should be likely to have high possibility producing specific antibody.

KNEDNIWFKRWKQD-C (15 mer) of HilA domain where it might be exposed to the outside in the three dimensional structure and GTDQKIDGDLKFDD-C (15 mer) of the FliC domain were synthesized. In further stage, the synthesized peptide was combined with carrier and injected into a rabbit. After the 3 times of boosting, the serum was obtained and the antibody was purified. Also *Salmonella* Mouse monoclonal antiybody, which is purchased from antibody production corporation, was used to make the bioreceptor.

In this research, bioreceptor is the kind of protein chip, and non-mark devices using the SPR to get the signal. Into the bargain, mark devices using the fluorescent substance were applied. SPR sensor chip, which is non-mark devices, comprised a thin gold film on the glass surface and immobilization of HilA and FliC antibody onto SAM, dextran layer was shown as the value of 3000 RU. Then, *Salmonella* sp. $1.0X10^8$ (CFU/ml) could be detected. Also, the immuno-fluorescent nanoparticles bound antibody which can be utilized as a mark devices for the detection of *Salmonella* were constructed through the immobilization of antibody onto the fluorescent PSQ nanoparticle comprising rhodamine as a fluorescent dye. As the results from the analyses with the formation of nanoparticle-antibody complex and with the reaction between this complex and pathogenic bacteria directly and onto the biosensor chip, defects of the photon immune sensor including irradiance and

specificity were overcome.

Through these researches, *Salmonella* HilA rabbit polyclonal antibody and *Salmonella* FliC rabbit polyclonal antibody can be applied to various biosensor. The manufacture technology of immunosensor chip and immuno-fluorescent PSQ nanoparticle could help to make immuno-biosensor, photon-immuno sensor and fluorescent immuno-sensor. Pathogenic bacteria infect host's body and cause illness, so through the biosensor, we need to find out them quickly and accurately if there were pathogenic bacteria to prevent illness.

References

[1] Kim YB, Kim YA, and Yoon KS *Macromol. Rapid Commun*, **27**:1247-1253 2006.

[2] PD Skottrup , M Nicolaisen, and AF Justesen *Biosensors and Bioelectronics,* **24**:339-342 2008.

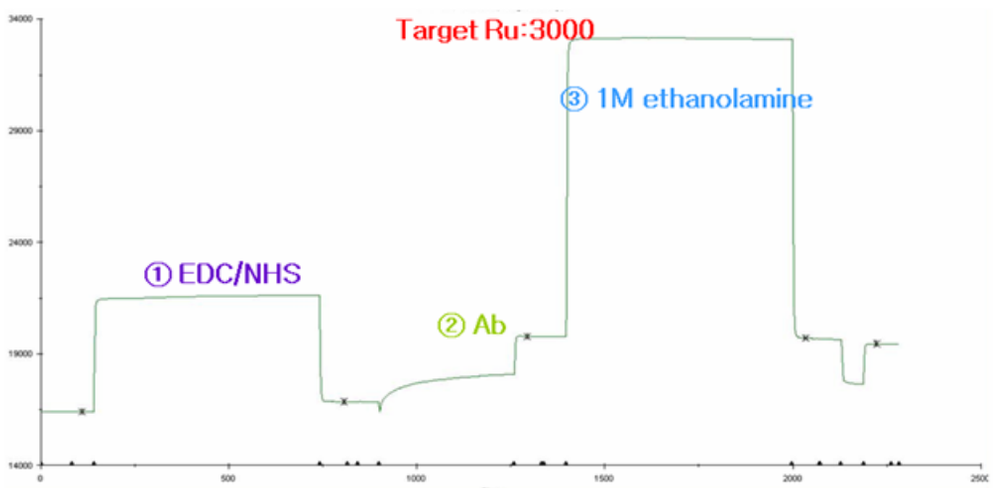

Fig. 1. The SPR sensorgram showing the procedure of HilA Ab immobilization on the sensor chip. A constant flow of PBS buffer (5 ㎕/min) was maintained the whole procedure of immobilization. ①. 35 ㎕ of 0.1 M NHS/0.4 M EDC, ②. 35 ㎕ of 50 ㎍/ml HilA, Ab in acetic acid buffer ③. 35 ㎕ of 1M ethanolamine (pH 8.5)

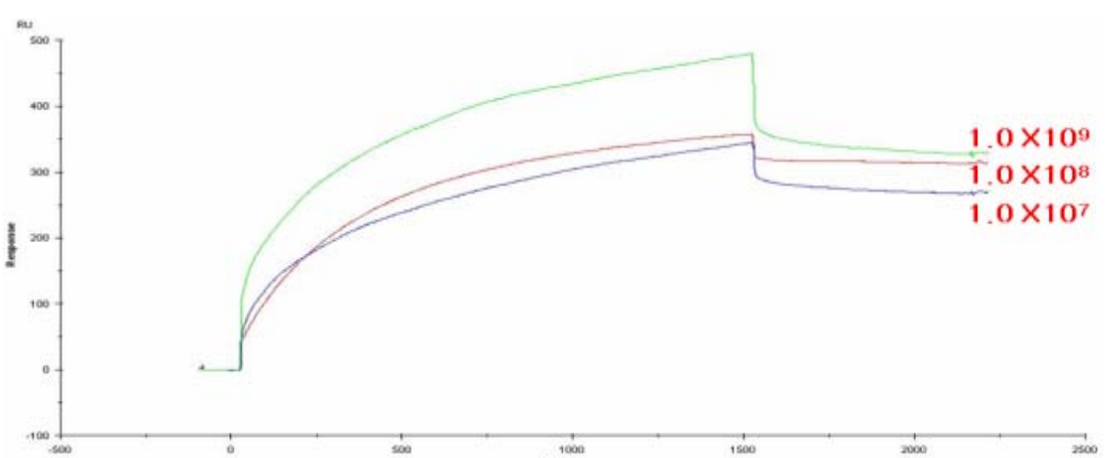

Fig. 2. Overlayed binding profiles in accordance with increasing concentration of *Salmonella* cells were passed over the sensor chip.

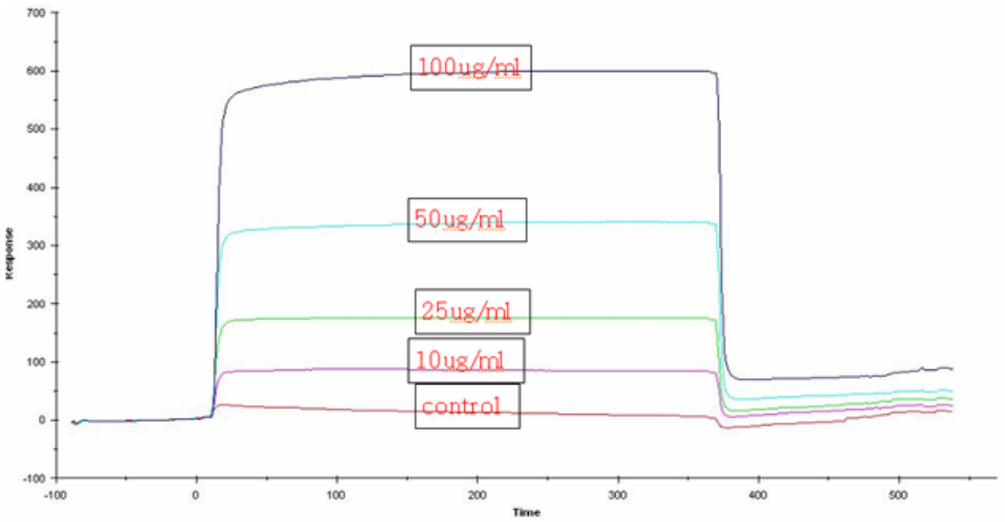

Fig. 3. Overlayed binding profiles in accordance with increasing concentration of HilA Ag were passed over the sensor chip.

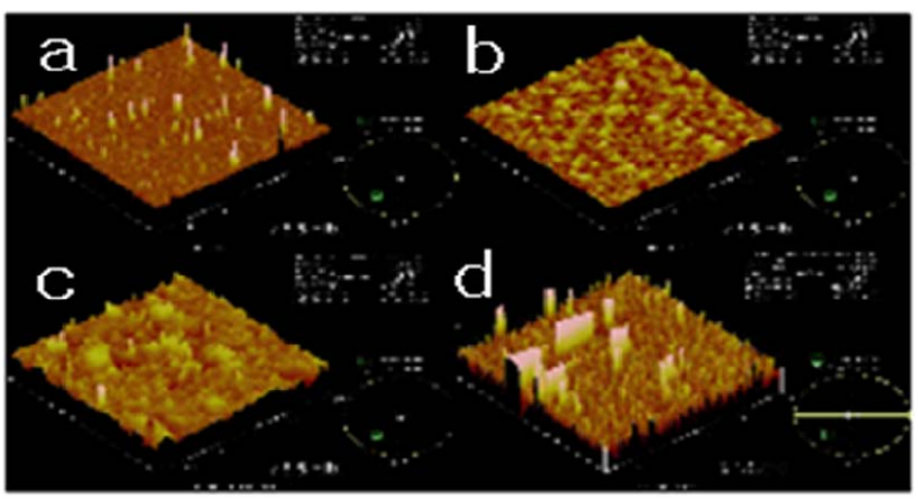

Fig. 4. AFM 3-dimensional images. A.bare Au, B. Self-assembled monolayer, C. HilA Ab against *Salmonella* on self-assembled monolayer, *D. Salmonella* on immobilized sensor surface.

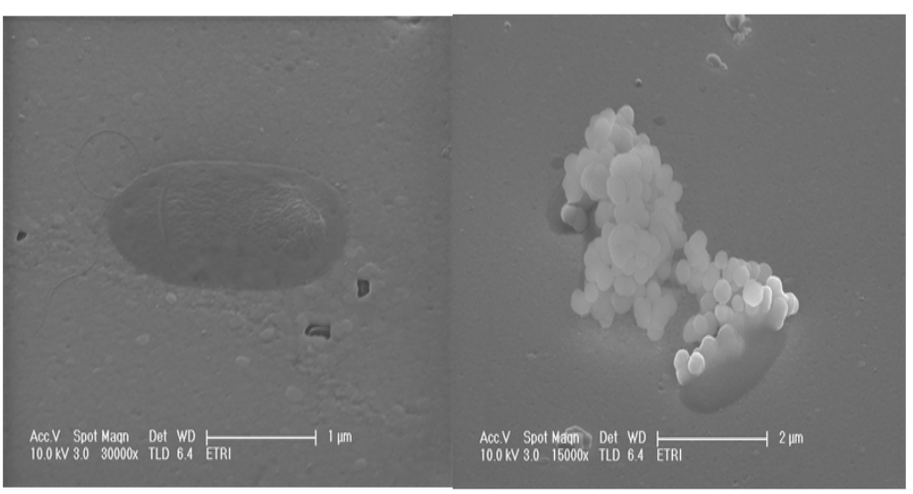

Fig. 5. SEM images of Bacteria – immuno nanopaticle interaction