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Isolation of *Listeria monocytogenes* by Immunomagnetic Separation and Atomic Force Microscopy

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***Listeria monocytogenes* is a pathogen of major concern to the food industry and the potential cause of severe infections such as listeriosis. Early detection of this foodborne pathogen is important in order to eliminate its potential hazards. So, immunomagnetic separation (IMS) has been suggested as a means of reducing the total analysis time and for improving the sensitivity of detection. Atomic force microscopy (AFM) has been used for measuring the topographic properties of sample surfaces at nanometer scale. In this study, we used AFM to confirm both the sensitivity and the specificity of IMS. Regarding AFM analysis, the length and the width of the bacteria, which were in agreement with literature values, were found to be 2.993 μm and 0.837 μm, respectively. As a result, AFM helped us both characterize and measure the bacterial and bead structures.**

Key words: *Listeria monocytogenes,* atomic force microscopy, immunomagnetic separation

Listeria monocytogenes is a widespread pathogen, which is of concern to public health and to the food industry, particularly during the last two decades, because it causes a high percentage of food-borne disease fatalities (Dominguez *et al.*, 1988). This pathogen is a causative agent of the disease listeriosis, which can take any one of three clinical forms in humans: i.e., encephalitis, septicaemia and abortion. Since *L. monocytogenes* can resist freezing and grow over a wide range of temperatures, including refrigerator temperatures, food products such as milk, cheeses, ice-cream, meats and ready to eat foods are potential major contamination sources for human listeriosis (Hsih and Tsen, 2001).

Since conventional culture methods are labor-intensive and 3 days is required to presumptively identify *L. monocytogenes* in a test sample, and up to 6 days is required to confirm the identity of the isolate, rapid isolation and identification procedures in food would enable us to increase the likelihood of preventing diseases caused by this pathogen (Ben Embarek, 1994; Hsih and Tsen, 2001). One such improvement involves the use of polystyrene particles called Dynabeads®, which are uniform, superparamagnetic, monodisperse polymer particles (Dynal, 2000). These beads consist of fine granules of iron oxide, which makes them superparamagnetic (Molla *et al.*, 1996). The use of these beads together with specific antibodies enables the sequestration of target bacteria from the contaminating microflora and thus the concentration of these bacteria into smaller volumes for further testing (Safarik *et al.*, 1995; Fratamico and Crawford, 1999). Thus, the term immunomagnetic separation (IMS) is used to describe this technique, which emanated from immunology, molecular biology and microbiology (Patel, 1994; Fratamico and Crawford, 1999). Due to these properties, IMS is becoming accepted as a new technique and has been used in many studies (Jacobsen *et al.*, 1997; Mercanoglu and Aytac, 2002). By using IMS, not only is the analysis time reduced, but also the sensitivity and specificity of microorganism testing can be increased.

Another technology that is applicable in microbiological studies is atomic force microscopy (AFM). AFM was introduced in 1986 (Binnig *et al.*, 1986), and since has been used to image a wide variety of biological materials, like bacterial cells (Umeda *et al.*, 1998), and human chromosomes (Ergun *et al.*, 1999). Basically AFM uses a probe mounted on a very small spring cantilever. When the probe is scanned across the sample, the force between the probe tip and the sample changes as the surface features are encountered, causing the spring cantilever to deflect. In order to obtain the magnitude of this displacement, a laser and a quadrant detector are used to detect the motion of the spring cantilever and provide a feedback signal as the scanning head is moved across the sample (Guven *et al.*, 1997). In this study, *L. monocytogenes* was

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visualized by AFM following its isolation from buffer solution by IMS.

Materials and Methods

Bacteria strain

L. monocytogenes (strain 1462) was obtained from the Institute for Milk Hygiene and Milk Technology, University of Veterinary Medicine, Vienna, Austria.

Immunomagnetic separation (IMS) technique and isolation

In IMS technique, Dynabeads® anti-Listeria (Dynal, Norway) has been used to isolate *L. monocytogenes* from buffer solution at approximately 10^2 cells/ml. Twenty µl of beads were incubated with 1 ml of this solution at room temperature for 10 min with continuous mixing (Dynal MX3; Dynal, Norway) so that the specific antibodies coated onto the beads would bind the target bacteria. The bead-bacteria complexes were subsequently separated using a magnetic particle concentrator (Dynal MPC-M®; Dynal, Norway). Afterwards, 1 ml of washing buf-fer (0.15 M NaCl, 0.01 M Na-phosphate buffer, 0.05% Tween 20; pH 7.4) was added to resuspend the beads and the separation steps were then repeated. Finally, 100 µl of washing buffer was added to resuspend the beads.

Atomic force microscopy (AFM) and analysis

The AFM used in this study was a TopoMetrix TMX2000 Explorer (Topometrix, USA), operating in contact mode in air. Throughout the surface analysis, a standard pyramidal tip (Topometrix 1520-00) with a radius of curvature of approximately 1000 A° and a 150 µm tripod scanner were used. These probes were made of Si_3Ni_4 and mounted on 200 µm long cantilevers.

Before spreading the samples onto the slides, the slides were ultrasonically cleaned. During the sample preparation, *L. monocytogenes* and the bead-bacteria complexes were spread onto glass slides. The unexposed beads were spread as a control. Next, the specimens were analysed by AFM, supported by line measure analysis (Topometrix, SPM Lab v3.06.06) software which was capable of measuring the dimensions of the images gathered by the AFM.

Various scan areas ranging from 100×100 µm^2 to 10×10 µm^2 were applied to image the specimen. The applied force and the image resolution were 0.7 nN and 400×400 pixels, respectively, for each image acquisition. The raw data gathered were analysed by using the software supplied with the atomic force microscopy system to provide two or three-dimensional patterns.

Results and Discussion

Following the implementation of IMS, the specimens

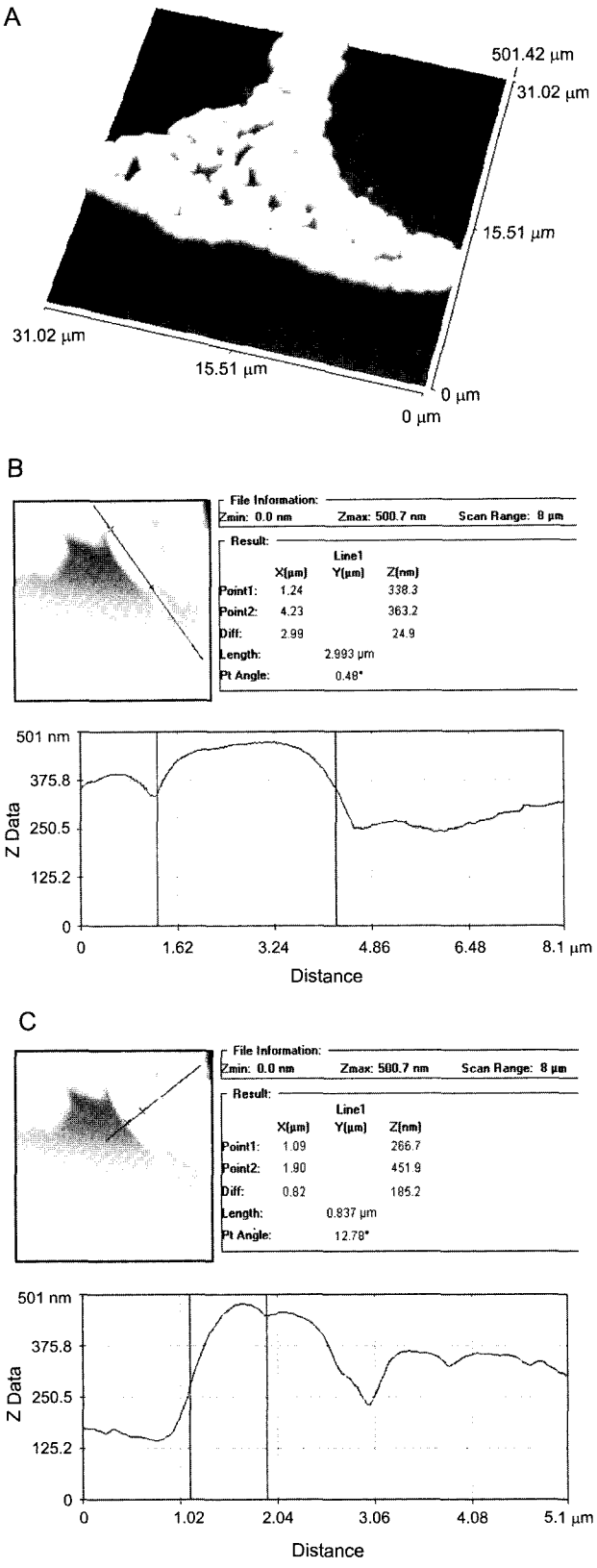

Fig. 1. A. The topographical image of *L. monocytogenes* B. Line measure analysis of *L. monocytogenes*. The length of *L. monocytogenes* was 2.993 µm. C. Line measure analysis of *L. monocytogenes*. The width of *L. monocytogenes* was 0.837 µm.

were prepared as follows; first specimens were prepared solely using *L. monocytogenes*, the second solely with beads, and the third using both *L. monocytogenes* and beads. In Fig. 1a, a topographical image of *L. monocytogenes* is shown. Line measure analyses are also shown in Fig. 1b and 1c, and indicate the dimensions of *L. monocytogenes*. The dimensions were found to be in accord with the known dimensions of this bacteria. The length of the bacteria was determined to be 2.993 μm and the width 0.837 μm. Fig. 2 shows beads, which were both visualised and measured. The measured bead diameter was 4.471 μm. Finally, images of the bead-bacteria complexes are shown in Fig. 3.

In this study, we used AFM, in order to investigate and to confirm the sensitivity and specificity of the IMS technique. IMS is a powerful technique for the detection of microorganisms and has high sensitivity and specificity. Using specific antibodies, only *L. monocytogenes* bound to the beads. On the other hand, AFM line measure analysis enabled us to measure the dimensions of bacteria, beads and bead-bacteria complexes. Bacteria dimensions

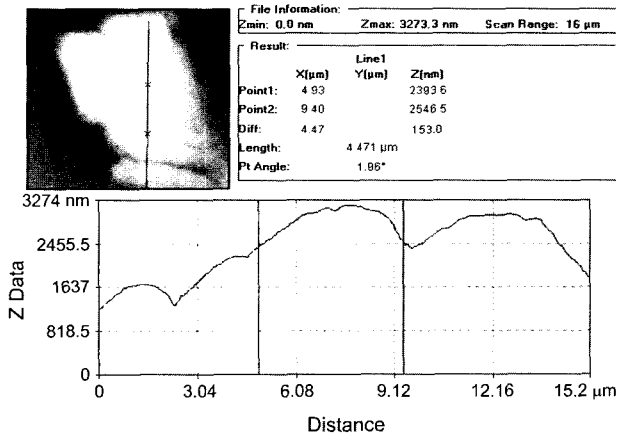

Fig. 2. Line measure analysis of anti-Listeria beads. *The bead diameter of the anti-Listeria bead was 4.471 μm.

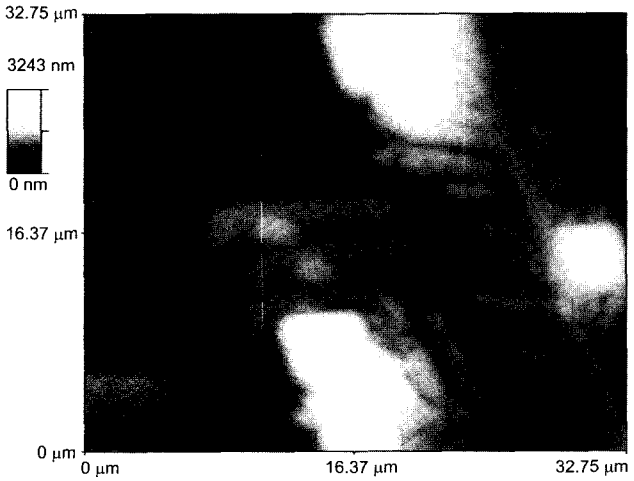

Fig. 3. The bead-bacteria complexes

were similar to the previous report that indicated the length and width of the bacteria as 0.5-10 μm and 0.5 μm, respectively (ICMSF, 1996). In addition, bead size (2.8 μm or 4.5 μm) was also in agreement with the stated size (Dynal, 2000). Finally, Fig. 3 shows the bead and bacteria complexes in order to confirm bead specificity by qualitative method AFM.

In conclusion, AFM and IMS combination provided a three-dimensional analysis, and allowed target bacteria capture, allowing us to determine the sensitivity and the specificity of the IMS technique. The image analysing capability of AFM helped us to interpret bacterial and bead structures, and demonstrated the interaction between the beads and the bacteria.

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