Abstract—Fundamental study on high speed detection of bio-particles (BPs) in air was carried out using discharge plasma. Fine particles in air were sampled on a collection plate effectively, using electrostatic precipitation. Collected particles were exposed to barrier discharge plasma to destroy the cell and to obtain cell lysates. In 20 sec, Escherichia coli and other cells can be destroyed. In situ electrophoresis in agarose gel and membrane filtering were examined to separate the DNA from the other substances in the lysates. The transferred DNA was stained with fluorescent dye for microscopic observation. In this experimental condition, enough amount of DNA for the detection was transferred through the thin agarose gel layer.

From the experimental results, detection of bio-particles in air can be detected by combining each step.

Keywords — bio-particles(BPs), electrostatic precipitation, dielectric barrier discharge(DBD), fluorescent microscopy, DNA

I. INTRODUCTION

Bio-particles (BPs), such as bacteria, viruses, fungi and others, cause health hazard, especially for those people with an impaired immune system. There is no definitive way to detect the danger of infection, partly because real-time monitoring of BPs is difficult. Conventional methods for identifying and enumerating such BPs rely on microscopic or cultural techniques and, as a consequence, are not suitable for quick detection for feedback control of bio-contamination. Recent advances in areas such as real-time PCR technology [1-6], micro-array technology [7-13], micro-electromechanical systems [14], micro-fluidics [15-17], and optoelectronics [18] present new technological possibilities for the detection of BPs that have DNA or RNA, however, the field of application of these technologies is limited because of its inherent complexity and the need for highly trained personnel for operation and interpretation of the results.

This report presents a new detection method of the BPs based on discharge plasma and the DNA detection technique in situ. In this study, E.coli and its bacteriophage lambda (λ) DNA are mainly used as model BPs and the potentiality of this system for reduction of detection time was investigated. We have proposed a system for rapid detection of BPs, as shown in Fig.1. The concept of this system consists of four steps, i) collection of BPs by electrostatic precipitation (ESP), ii) break the cell wall of the BPs using the dielectric barrier discharge (DBD) plasma, iii) extract the genomic DNA and transfer to the Nylon membrane using electro-blotting. iv) detect the genomic DNA. We have investigated these steps separately.

II. CONCEPT AND EXPERIMENTAL PROCEDURE

A. Collection of BPs

In the detection system, use of tape as the reaction substrate has been proposed for continuous operation. Gel is coated on the tape so air cannot penetrate, therefore, conventional filter sampling methods are difficult. In order to collect BPs effectively on gel or film, we used the electrostatic precipitation (ESP), as shown in Fig.2. YPD medium modified surface was used as a ground electrode to collect suspended BPs. A needle electrode made of silver wire (0.4mm φ) for reducing ozone production energized with DC high voltage was used. The electrode separation was 40 mm. The reactor...
was placed in a box and room air was supplied using a fan (flow rate; 18 L/s). To evaluate the BPs collection, the YPD medium plates (9cm φ) were incubated at 30°C for 48hr for colony forming.

In order to compare the collection efficiency, we counted microbes after cultivation. In the proposed system, DNA in BPs are extracted, filtered, stained, and counted, therefore, collected BPs need not to be viable.

B. Breaking the cell wall of BPs

DBD is very effective for sterilization of the surface of plastic film and other materials. With 40 sec exposure to DBD at atmospheric pressure, *Bacillus Subtilis* spores can be destroyed to less than 10⁻⁶ viability [19]. The main cause of the damaging of living cells by DBD has been considered to be physical destruction of their cell wall caused by streamer or chemical damage by reactive species [20]. In the rapid detection system, extraction of DNA from cells is necessary. Usually an enzyme is used for this process, however, it takes time. From the DBD sterilization experiments, cells are punctured. This result suggests that DBD cell destruction could be used for rapid extraction of DNA.

Fig.3 shows the dielectric barrier discharge (DBD) reactor, consisting of parallel electrode with insertion of an insulating plate. Electrode spacing was 2 mm. With the AC application, filamentary streamers are generated in the electrode spacing. The peak to peak voltage, the frequency of the applied voltage and the input power were 25kVpp or 40kVpp, 2kHz, 20W.

In this study, *E.coli* transformant MV1184(pGLO) was selected as a sample. The transformants produce and accumulate Green Fluorescent Protein (GFP) in their cytoplasm which causes them to glow a brilliant green color under ultraviolet light. The bacterial cells were grown at 34°C with shaking in LB medium (10g bacto-tryptone, 5g bacto-yeast extract and 10g of NaCl in 1L of MilliQ water, pH 7.0) supplemented with 4mM L-arabinose which induces GFP expression. 10ml of the overnight culture was centrifuged at 4,000 × g for 5min at room temperature. The pellet of the cells was resuspended with 10ml of distilled water. 0.1ml of the *E.coli* suspension (5.3x10⁹ cells/ml) was spotted and spread on the slide glasses coated with 0.2% gelatin in advance, then dried at room temperature. After DBD treatment, the cells were recovered using 0.2ml of 10mM Tris-HCl (pH8.0), 1mM EDTA into microtubes by repeated stroke of the solution with micropipette. These recovered cells were rendered for microscopic observation, measurement of fluorescent intensity and DNA analysis by agarose gel electrophoresis.

C. Genomic DNA transfer and detection

Airborne particles collected using ESP contain considerable amounts of dust which does not derive from biological materials. One effective method to distinguish BPs and non-BPs is to stain chromosomes of BPs with DNA specific fluorescent dye [22]. But in this case, some kind of dust reacts with the dye which disturbs the enumeration of BPs. The thin agarose gel will solve this problem by filtering the cell debris and other unnecessary elements, and separating DNA molecules from BPs (Fig.1). Using electro-blotting, separation of DNA was tested.

For the blotting experiment, *E. coli* genomic DNA was prepared by phenol extraction and ethanol precipitation after the cells were lysed at 70°C in the presence of 1% SDS. λ phage DNA was purchased from Takara Biomedicals. These genomic DNA were also used as hybridization probes, which were heat-denatured and labeled by random primed incorporation of digoxigenin-labeled deoxyuridine-triphosphate
(Dig-dUTP) during DNA synthesis using Klenow fragment.

To test the DNA transfer, different amounts of lambda-DNA and *E. coli* genomic DNA were spotted on a QMA filter (Quartz Membrane filter: Whatman 1851047). QMA filter is very stable in wet condition and is easy to handle. Before spotting the DNA, the filter plate was immersed in 1× TBE (89mM Tris-borate buffer (pH8.3), 2mM EDTA). Nylon membrane filter (Zeta-Probe membrane, Bio-Rad) was used to transfer the DNA on its surface. The DNA-spotted filter paper and the nylon membrane were used for the electro-blotting, according to CriterionTM Blotter (Bio-Rad) instruction manual. 1% agarose gel (5mm thickness) was placed between them. This agarose gel is used for filtrating the cell debris and other unnecessary elements. To check the DNA transfer efficiency, the electro-blotting was carried out in 1× TBE buffer at 20V overnight at 4°C.

After the electro-blotting, the transferred DNA was denatured with alkali, and quantified by DNA-DNA hybridization using a non-radioactive probe (Dig DNA labeling and detection kit, Roche applied science). The hybrid DNAs are detected by enzyme-linked immunoassay using an anti-digoxigenin alkaline phosphatase conjugate and subsequent phosphatase catalyzed color reaction with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium salt.

III. RESULTS AND DISCUSSION

A. Collection of BPs by ESP

The effect of ESP for BPs collection was investigated. Particles in room air were collected on a petridish for 10min. Fig.4 shows that the number of collected bacteria increased with applied voltage. Positive and negative corona discharge shows that the number of collected BPs increases with the increase of applied voltage. Positive corona discharge shows a higher colony formation than the negative one. This result is due to reduced ozone production in positive corona, killing less BPs on the culture media. The highest collection efficiency was observed with applied voltage of +25kV. In this condition, collection of bacteria was enhanced by about 50 times compared with no voltage application.

In addition, applied voltage and the distance between electrodes affected the collection of BPs. As closing to the center of the plate that was beneath the high voltage electrode, the number of colonies increased (data not shown). The colonies at the center decreased when very high voltage ( >30kV) was applied, because of the harmful effect on living cells by the generated ozone, their ions, and radicals.

B. Destruction of *E. coli* by DBD

Sometimes, destruction of cells by exposure to DBD could be due to small punctuation of cell walls. In this case, it is difficult to detect the slight damage of cells by microscopic observation. So, we used the GFP accumulating in *E.coli* cells as a sample to apply DBD.

![Fig. 5. DBD treated E.coli producing GFP.](image-url)
Blight GFP in cytoplasm of these cells might work as an indicator of the damage on the cell wall by leaking outside the cell through the pores. Fluorescent microscopy images of E. coli cells recovered from slide glasses are shown in Fig. 5 with and without the exposure to the DBD. Originally the fluorescence was very bright, and the cells are clearly observed. There was a decrease in the fluorescent intensity of the cytoplasm with the time of exposure to DBD. Two types of explanation were possible for the cause of decrease in the cytoplasm fluorescence. One is the dilution of the GFP in cytoplasm by leaking it outside the cell through the pores, and the other is the bleaching of GFP itself. Fluorescent spectroscopic analysis of each recovered solution indicates the latter was the case (Fig. 6). Bleaching of GFP, especially those with the long exposure time to DBD, was observed. The bleaching means that DBD leads to denaturation or degradation of proteins even if the size of the target for discharge plasma is very small.

We have also investigated the DNA state. The recovered samples were centrifuged at 8,000 x g for 10 min and divided into fractions of supernatants and precipitates. DNA was extracted by heating at 70°C in the presence of 1% SDS from the supernatants or the precipitates, and analyzed by 1% agarose gel electrophoresis (Fig. 7). In this condition of the electrophoresis, the chromosome DNA was separated into two different positions on the gel. One migrates similarly to 23kb DNA fragment of the size marker and the other stays near the sample well which does not enter the gel matrix because the size is larger than the migrated one. Plasmid pGLO DNA (5.4kb) is also seen at the lower band as super-coiled and the upper band as relaxed form [21].

Analysis of supernatants shows that DNA is released from the cells to the outside by DBD treatment, suggesting that breakage of the cells has occurred. At 10s, exposure to the discharge, some large DNAs are seen near the sample well, and the longer exposure time to the discharge causes gradual degradation of these DNAs. Analysis of precipitates shows that the DNA inside the cell or cell debris remains near the sample well, indicating a larger size compared to that of the supernatants even after the longer exposure time to DBD.

The major component of the plasmid DNA seen in the supernatants is relaxed [21], which is injured by nick introduction. In precipitates, on the other hand, the majority of the plasmid is a covalently closed circular form. These results also suggest that the reactivity of the electric field of DBD is stronger outside of the cell than inside of the cell. The length of this DNA seems enough for further in situ experiments which prefer DNA larger than thousands of nucleotides.

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**Fig. 6.** Fluorescence of DBD applied samples.

**Fig. 7.** 1% agarose gel electrophoresis of the DBD applied samples.

**Fig. 8.** DNA transfer from the QMA filter to Nylon membrane.

a) QMA filter spotted different amount of λDNA. Bromophenol blue is included to the DNA solution to visualize each spot.

b) Nylon membrane after color development of transferred λDNA.

c) QMA filter spotted different amount of E. coli DNA.

d) Nylon membrane after color development of transferred E. coli DNA.
C. Electro-blotting of DNA

Fig. 8 shows the results of electro-transfer of the DNA from QMA filter to Nylon membrane. After the transfer, a known amount of the DNA was spotted as a control on the margin of the Nylon membrane. DNA on the Nylon membrane was hybridized with the probes of DNA which share the same sequence labeled with digoxigenin. The digoxigenin was detected by enzyme-linked immunoassay and color reaction. We estimated the amount of transferred DNA by comparing with control DNA using the image processing software (ImageJ). The results show that 33% (on average) of DNA could be transferred. The E. coli genomic DNA is considerably longer in size than λ DNA, however significant amounts (15%) of genomic DNA can be transferred to the nylon membrane, enabling the enumeration the BPs.

IV. CONCLUSION

Electrostatic precipitation with an applied voltage of +25kV enhanced the collection of the bacteria in air about 50 times compared without voltage application. Using the atmospheric pressure DBD, DNA can be extracted. In the meantime, the result suggested that DBD did not severely damage the cellular DNA. The electro-blotting results suggest that DNA can be filtered and transferred to the nylon membrane for the fluorescent detection. From these experimental results to confirm each steps, combination of these steps will make it possible to realize real-time or fast detection system of BPs. The authors will report in the future on the continuous progress of this detection method.

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