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Characterization of DNA damage caused by atmospheric cold plasma using bacterial DNA repair machinery

大気圧低温プラズマによる細菌DNA損傷のDNA修復機構を指標とする評価

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A comparison between sensitivity to plasma exposure in acidic solution of three bacterial strains was carried out. Radiation-resistant bacterium (*D. Radiodurans* R1), two strains of *Escherichia coli*, one has deficaty in DNA repair pathway and the other does not. *D. rad* has DNA repair ability stronger than common, radiation-sensitive bacteria. Evaluation of efficacy of plasma inactivation for radiation-resistant bacteria, and weight of DNA damage was tried. Exposure to high temperature in acidic solution was also carried out for comparison. In result, *D. rad* showed low sensitivity to plasma exposure. Contrastingly, it showed high sensitivity to high temperature.

1. Introduction

Efficacy for inactivation using atmospheric cold plasma (plasma) to radiation-resistant bacteria was investigated.

Two strains of *Escherichia coli* (*E. coli*) were also treated by plasma. And three bacteria were also exposed to high temperature as stress of different type. By comparison between the bacterial species (strains) and type of stress, evaluation of DNA damage in plasma inactivation was tried in parallel way.

Three bacteria, *Deinococcus radiodurans* R1 strain (*D. rad*), *E. coli* ATCC13706 strain (ATCC1306), and *E. coli* MV1184 strain (MV1184) were used. *D. rad* is the most common radiation-resistant bacterium. It has the far stronger DNA repair machinery than common radiation-sensitive bacteria, according to the fact that radiation inactivates organism by inability of growth by gene defective. [1] *E. coli* was chosen as common, radiation-sensitive bacterium. MV1184 has a few defective genes for its usability as carrier of artificial foreign DNA. *recA* is one of these genes and has major ability in its repair pathway of damaged DNA.

It is known main factor of cell inactivation by heating is denaturation of protein. The main factor of plasma inactivation in air is oxidation by radicals, though main target is not clarified.

The plasma used in this research could not inactivate bacteria enough, at neutral pH (data not shown). Though, increase of strength of plasma treatment was avoided to keep affinity with application to human body. Instead of it, enhancement of bactericidal effect with combination with acidic environment, which is explained by conversion of superoxide anion radical (O_2^-) to hydroperoxy radical (OOH) was used. [2]

2. Experimental procedure

6.1 *Atmospheric cold plasma source*

Atmospheric cold plasma jet [3] was used as plasma source. A schematic diagram of plasma jet and parameter are shown in Fig. 1.

6.2 *Preparation of bacterial sample*

Each bacterium was streak cultured on nutrient agar to obtain single colony. Seed culture was prepared by inoculating the single colony to 20 ml nutrient liquid medium, and pre-cultivating. 1/100 volume of the seed culture was inoculated to fresh nutrient medium, and cultivated. Cultivation temperature was 32°C for *D. rad* and 37°C for the two *E. coli* strains. And cultivation time was 48 h and 24 h.

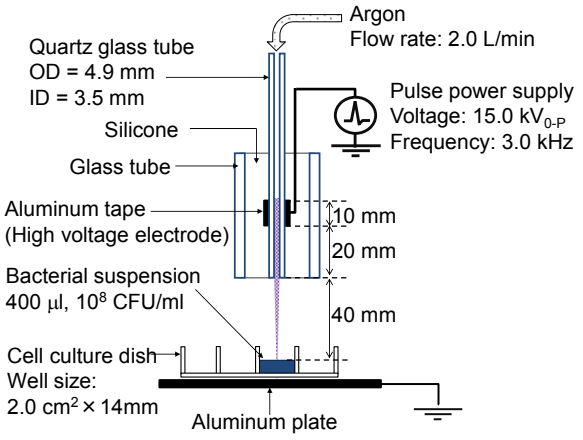

Fig. 1. Schematic diagram of plasma jet generator

The bacterial culture was centrifuged at 2600 rcf for 10 min. The precipitated bacteria were resuspended to same amount of 10 mM, pH 3.8 sodium citrate buffer (pH 3.8 buffer). And the bacterial suspension was centrifuged again. Then, washed bacteria were resuspended to pH 3.8 buffer. The bacterial concentration was adjusted to 10^8 CFU/ml.

6.3 *Exposure to atmospheric cold plasma*

400 μl each of the bacterial suspensions was injected into a cylindrical well of cell culture plate. Plasma jet generator was fixed at the distance 40 mm from surface of bacterial suspension.

The parameters of the cold plasma jet are described in Fig. 1.

3 μl of the bacterial suspension was harvested after 0 sec (Control), 30 sec, 1min, 2min, 3min, and 4 min exposure.

The harvested suspension was diluted in 300 μl of fresh pH 3.8 buffer to stop chemical reaction. Then, concentration of viable bacteria was measured by standard plate count.

6.4 *Exposure to high temperature*

65°C was used as the high temperature, because it showed the survival rate resembling the plasma exposure. Bacterial suspension was adjusted 10 times higher than it in the plasma treatment. And 30 μl of bacterial suspension was added to pre-warmed, 270 μl pH 3.8 buffer.

3. Results and discussion

Fig. 2 shows the result of plasma exposure in acidic condition. The decrease of survival rate along treatment time indicates bacteria's sensitivity to the each of stresses. *D. rad* showed far lower sensitivity to plasma than both *E. coli* strains. Fig. 3 shows the result of heat exposure. *D. rad* showed high sensitivity to heat treatment.

The both *E. coli* strains did not show a substantial change between plasma and heat, contrasting to *D. rad*. Unexpectedly, ATCC13706 showed higher sensitivity to both treatments than MV1184, despite of defect in DNA repair gene in the latter. MV1184 has a few defective genes other than the major gene in its DNA repair pathway (*recA*). Though, they seem to have little effect on plasma sensitivity, according to their clarified functions. It seems difference between two *E. coli* strains other than the defects of genes was much greater than the expectation.

4. Conclusion

- *D. Rad* in acidic solution showed low sensitivity to plasma exposure and contrastingly, high sensitivity to high temperature.
- The *E. coli* strain with defect in DNA repair pathway was not more sensitive.
- *E. coli* showed similar trend in both plasma and heat treatment.

References

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[2] S. Ikawa, K. Kitano and S. Hamaguchi: Plasma Process. Polym. 7(2010) 33.

[3] M. Laroussi and X. Lu: Applied Physics Letters. **87** (2005) 113902.

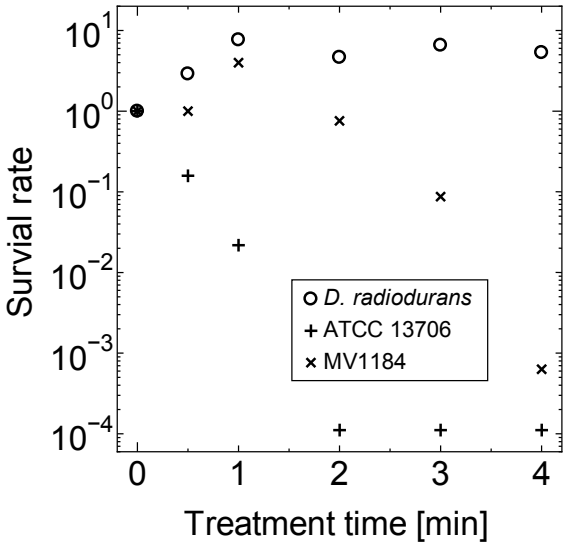

Fig. 2. Survival rate after plasma exposure

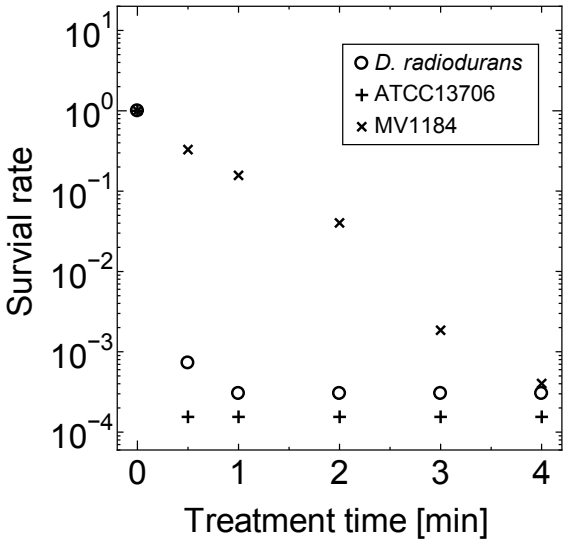

Fig. 3. Survival rate after exposure to high temperature (65 °C)