Inactivation mechanism of spore-forming bacteria by plasma of parallel plate electrode-type DBD

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Abstract: Influence of the plasma produced by atmospheric pressure barrier discharge on spore-forming bacteria is investigated. Activity of the bacteria is evaluated by the growth characteristics and the gene expression analysis, after being irradiated with the barrier discharge. Spore-forming bacteria irradiated with dielectric barrier discharge had a 23% reduction in relative growth rate compared to the unirradiated sample. Some responses to active oxygen appear on the gene profile of the gene, which lead to inactivation mechanism, expression analysis.

Keywords: Plasma, spore-forming bacteria, DBD.

1. Introduction

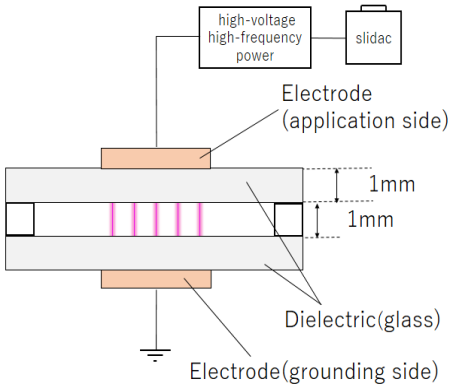
The plasma sterilization technique is focused in the field of agriculture, medicine and food. Many studies are in progress to inactivate and sterilize bacteria by using plasma. Low temperature treatments are achieved, which lead to prevention of protein damage. Although there are many researches applying inactivation of bacteria by plasma, inactivating factors and the mechanism of killing bacteria by plasma have not been sufficiently elucidated. When the major inactivation factors in the plasma against the bacteria are specified, it can lead to various effects of the sterilization process such as shortening of sterilization time, reduction of gas amount used for sterilization.

Bacteria are classified into two types of prokaryotes and eukaryotes from the difference in structure. In this study, to describe the effect of plasma on the spore of *G.stearothermophilus* belonging to prokaryotes.

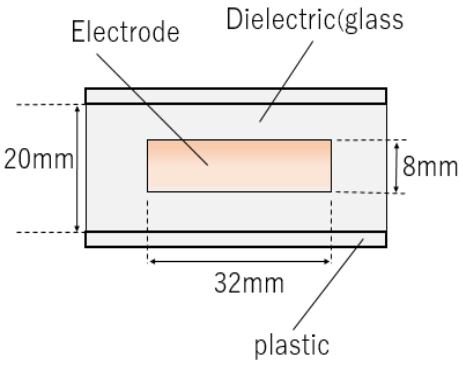
2. Experimental method

The schematic diagrams experimental apparatus and the actual size are shown in Fig 1. Parallel plate electrode-type dielectric barrier discharge was used for the plasma irradiation. The gap between the dielectrics is 1 mm in gap distance and 20 mm in width, and gas is introduced into this gap. High-voltage (~10kV) and high-frequency (~10kHz) power source is used as a power source. The spore-forming bacteria was placed in the gap between the dielectrics, and plasma was produced flowing gas into the gap. Plasma irradiation conditions are shown in Table 1. After irradiation, spore-forming bacteria were cultured in 3mL liquid medium at 58 °C. Thereafter, the absorbance (OD600) of the medium was measured with a spectrophotometer every one hour (every 30 minutes in the logarithmic growth phase).

Gene expression analysis of plasma treated spore-forming bacteria is performed to investigate the effect of plasma on bacteria in detail. Sample of bacteria used for analysis is nutrient-state bacteria during logarithmic growth phase.



Side view (axial)



Top view

Fig.1 Schematic diagrams of experimental device

Table.1 Plasma irradiation conditions

Gas	Oxygen and Argon
Voltage(pk-pk)	pk-pk 17.0[kV]
Gas flow rate	1[L/min]
irradiation time	0,60s,120s
Cultivation period	0~14h
Bacteria(spore type)	G.stearothermophilus(1.4×10^6)

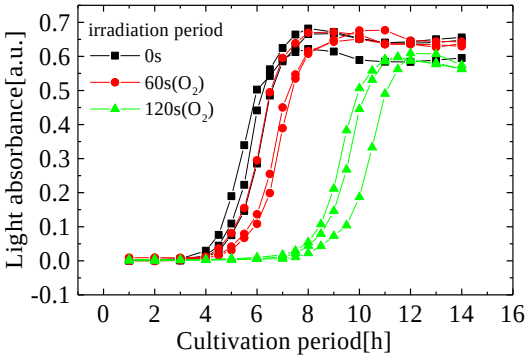


Fig. 2 Growth characteristics curve of bacillus irradiated with oxygen plasma

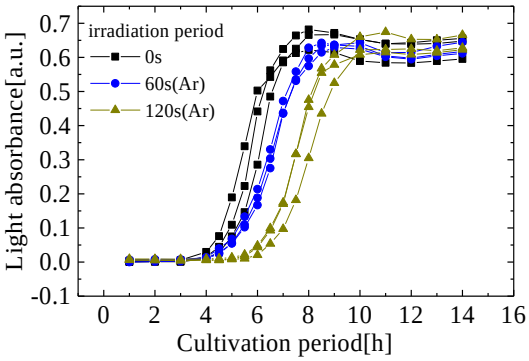


Fig. 3 Growth characteristics curve of bacillus irradiated with argon plasma

3. Results and discussion

3.1 Growth characteristic of the bacteria

Results of the growth characteristic of the bacteria varying the irradiation period of oxygen plasma and argon plasma are shown in Fig. 2 and 3. the light absorbance by bacillus in culture medium is observed, which is proportional to the number of bacillus, changing the culture period. The plasma irradiation period is set at 0, 60, 120 sec. Absorbance is 600 nm, which correlates well with the amount of bacteria.

From the results in Fig. 2 and 3, in both cases of the oxygen and argon plasmas, the initiation of the growth bacteria is delayed depending on the plasma

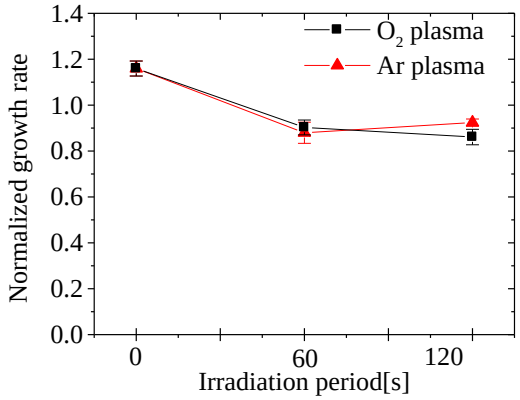


Fig. 4 Normalized growth rate varying the irradiation period

irradiation time.

Figure 4 shows the normalized growth rates of each logarithmic growth phase changing the plasma irradiation period of oxygen and argon plasmas. The normalized growth rates are calculated using a least-squares method from a linear graph obtained by taking logarithmic ordinate of the logarithmic growth phase as a logarithm.

Treatment temperature of oxygen plasma is below 60 °C, treatment temperature of argon plasma is below 70 °C.

3.2 Gene expression analysis

Figure 5 shows typical sequence of gene expression processes of bacteria that obtained from gene expression analysis. This result is strongly expressed in both parameters that oxygen and argon gas. It is a nucleotide synthesis process from uracil bases in RNA (ribonucleic acid) [(1)]. The nucleotide is called Uridylic acid (UMP). Nucleotides are generated from *de novo* synthesis of base, pentose and phosphate. The nucleotides are phosphodiester bond, they become DNA and RNA. Figure 6 shows UMP. UMP is one of a nucleotide unique to RNA. Nucleotide is composed of Base and Pentose, Phosphoric acid. In this structure, when the base is uracil, the nucleotide is UMP [(1)]. When Synthesis of UMP is delayed, synthesis of RNA necessary for protein synthesis is also delayed. In this case, it is inferred that increasing the period to synthesize ingredient that is necessary for DNA replication and cell division. As a result, it is thought the growth rate of bacteria decrease.

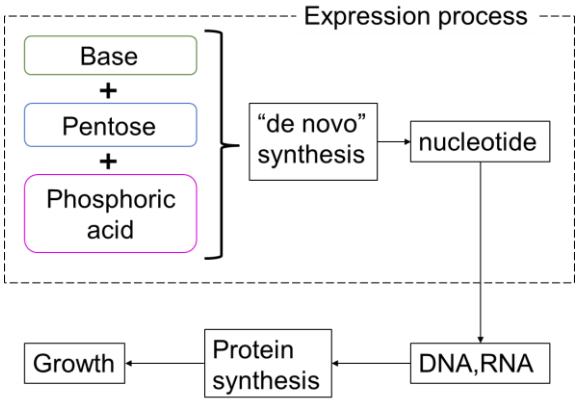


Fig.5 Expected route from plasma irradiation to growth of bacteria

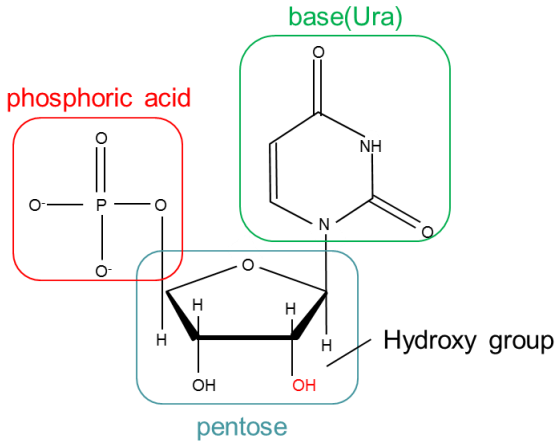


Fig.6 chemical structures of uridylic acid (UMP)

4. Conclusion

Spore bacteria were irradiated with dielectric barrier discharge by oxygen gas and argon gas, and the growth characteristics of the bacteria were measured. From this growth characteristic, when irradiated with plasma, germination is delayed and a decrease in growth rate was observed. The spore-forming bacteria that has been irradiated with plasma is delay to produce UMP. Delayed UMP production also delays RNA synthesis. As a result, the relative growth rate of bacteria was decrease. It is inferred that oxygen plasma and argon plasma have one of the factors that inactivate spore-forming bacteria.

5. References

[1] Bruce Alberts, essential cell biology, 2nd ed, Garland science, (2003)