RES-028

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Bactericidal effects of high-speed water nanodroplet technology against spore-forming pathogens

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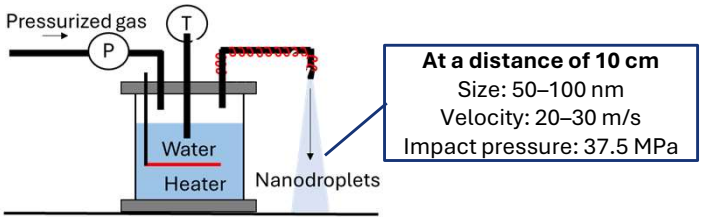
Background

Spore-forming bacteria, including *Clostridioides difficile* and *Bacillus anthracis*, are public health threats. In 2017, we developed a high-speed nanodroplet generation technology using water only as a new sterilization method. This technology produces droplets without wetting the contact surface. In this study, we evaluated the bactericidal effect of nanodroplets on the spores of *Clostridium* and *Bacillus* species.



P: pressure gauge, T: temperature meter. The impact pressure of nanodroplets colliding with a bacterium is 3.75×10^7 Pa (37.5 MPa) at a distance 10 cm.

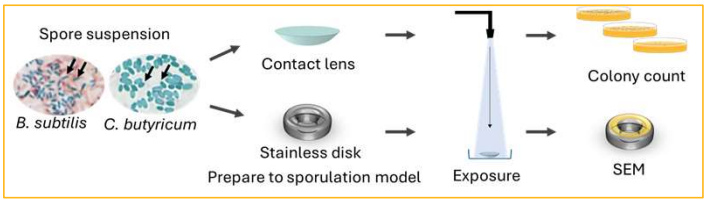
Methods

●Bacterial strains and sporulation

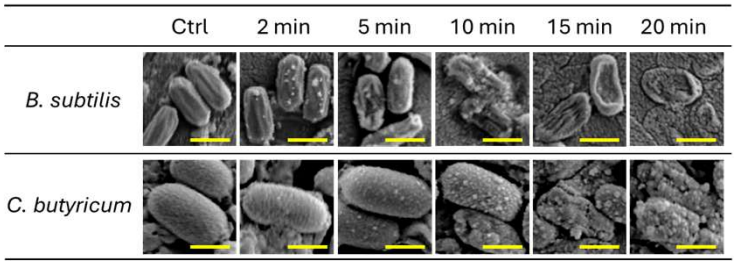
Bacillus subtilis ATCC6633 spores were prepared in difco sporulation medium at 37℃ for 3 days with shaking. The spores of *Clostridium butyricum* were obtained after anaerobic incubation on Brucella HK agar at 37℃ for 5 days and suspended in milli-Q water. Sporulation was confirmed by Schaeffer-Fulton stain.

●Impact of high-speed nanodroplets on each spore

After exposure to nanodroplets for 2–20 minutes at 37.5 MPa, morphological changes were observed by scanning electron microscopy (SEM), and viable counts were measured.



Results



(Bar, 1 μm, Magnification: × 15,000)

Fig. 1 SEM images of spore-forming
After 2 minutes of exposure to nanodroplets, projections appeared on *B. subtilis* spores, which dented over time and flattened by 15 minutes. In *C. butyricum*, projections were observed at 2 minutes, with holes forming at 10 minutes.

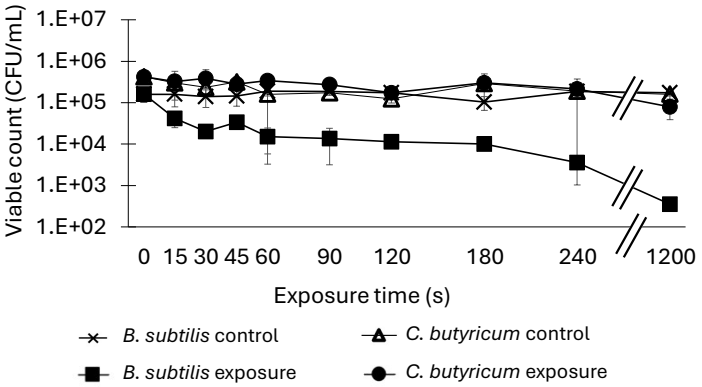


Fig. 2 Changes in viable count over time
After exposing nanodroplets for 20 minutes, *B. subtilis* decreased to 3.53×10^2 CFU/mL, while *C. butyricum* remained at 7.87×10^4 CFU/mL.

Conclusion

B. subtilis spores were flattened and killed under an impact pressure of 37.5 MPa, whereas damage to *C. butyricum* spores was limited. These results suggest that *C. butyricum* spores possess a more robust structure than those of *B. subtilis*.

References

1. Xiao Y, et al. Int J Plasma Environ Sci Technol 16:e03003, 2022.
2. Tamura Y, et al. J Bacteriol 206:e0013924, 2024.

Disclosure Statement of COI

The authors have no financial conflicts of interest disclose concerning the study.