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惑星保護（Planetary Protection）活動の現状と生物汚染の低減に関する取り組み

Current status of planetary protection activities and studies on bioburden reduction.

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1. Introduction

Planetary Protection is the practice to prevent microbial contamination of the solar system by spacecraft (forward contamination) and extraterrestrial contamination of the Earth (backward contamination). Space exploration missions are promoted to comply with the Planetary Protection Policy and Requirements established by the Committee on Space Research (COSPAR) to ensure that future scientific investigations related to the chemical evolution of the solar-system bodies and the origin and distribution of life are not compromised[1, 2]. The microbial contamination (bioburden) of flight systems, including landers or rovers, must be below the acceptable limit, especially for missions to Mars, Europa, Enceladus, and other solar-system bodies where the existence of extant life cannot be excluded. We have showed our studies on bioburden reduction this article. The related activities of TansaX (JAXA) for planetary protection will be introduced in the presentation.

2. Techniques for Reduction of Bioburden (Microbial Contamination)

Several sterilization methods are available for bioburden reduction, each with different mechanisms of effect and with advantages and disadvantages. Conducting a comparative study on sterilization methods against different types of microorganisms is essential to select appropriate techniques for bioburden reduction: Dry heat has been the standard method for since the Viking lander[3, 4]. However, this method does not apply to non-heat-resistant materials such as electronics[5]. Ultraviolet (UV) irradiation is a powerful sterilization method, but it can cause component surface degradation and cannot reach shaded areas. Alcohol disinfection is commonly used for bioburden reduction during spacecraft assembly, such as for the Mars Science Laboratory or Mars2020; however, alcohol wets the object and disinfect only the surfaces. Treatment with aqueous solution and vapor of hydrogen peroxide (H_2O_2) is a non-residual method because it degrades into water and oxygen. However, this method requires a high chemical concentration and may alter the material.

Plasma sterilization has the advantages of no residuals, no chemicals, and minimal adverse effects on components; however, quantitative knowledge is still limited, so it has not been applied for spacecraft bioburden reduction.

3. Comparative Study of Bioburden Reduction techniques using Several Microbes

In order to select the suitable sterilization techniques to bioburden reduction for forward planetary protection, we compared the effects of dry heat, UV light, IPA (isopropyl alcohol), H_2O_2, VHP (vaporized hydrogen peroxide), and oxygen and argon plasma on different types of microbes in this study.

This study selected important microbes according to several previous studies on planetary protection[6-8]. *Bacillus atrophaeus* is a Gram-positive bacterium, and its endogenous spores are widely used to test dry heat, ethylene oxide, steam and radiation. *Deinococcus radiodurans* are Gram-positive, vegetative bacterium with known radiation and desiccation-tolerant profiles. *Brevundimonus diminuta* is a Gram-negative bacterium and is a standard organism for validation of sterilizing-grade membrane filters. and *Aspergillus niger* is a filamentous fungus and its spores are highly pigmented, resistant to UV-C radiation, and easily dispersed through air. The possibility of these microorganisms attaching to the spacecraft during the pre-launch process is worth assuming. Spores and cells were air-dried on glass or metal plates to simulate microbial contaminants on the surface of spacecraft and used for each sterilization test. Here, a colony counting method, a gold standard method for the sterilization tests, was used for the evaluation of living organisms after microbial reduction treatment.

4. Results and Discussion

Table 1 summarizes the treatment times required to achieve sterilization which means survived cells were not detected. *B. atrophaeus* showed highly resistance to dry heat, 70% IPA, 7.5% H_2O_2 and VHP compared to

Table 1. Required time to sterilize microbes by using dry heat, UV irradiation, exposure to antimicrobial agents including VHP and plasma treatment (Revised from Kimura et al., under review). ND: no data.

Treatment		Microorganism			
		***Bacillus atrophaeus* NBRC13721 (spores)**	***Deinococcus radiodurans* NBRC15346**	***Brevundimonas diminuta* NBRC14213**	***Aspergillus niger* ATCC16888 (spores)**
Dry heat *	Treated in an oven kept at 120ºC	75 h	<1 h	<0.5 h	<0.5 h
	Treated in an oven kept at 140ºC	5 h	<0.5 h	<0.5 h	ND
Ultraviolet irradiation	Exposure to UV-light (253.7 nm) at a dose of 1.26 J/cm^2 per hour	<8 h	30 h	<8 h	Incomplete (treated for 30h)
Antimicrobial agents	70% Isopropyl alcohol (IPA), treated for 10 min	Incomplete	<5 min	<5 min	<5 min
	7.5% Hydrogen peroxide, treated for 10 min	Incomplete, but damaged †	<5min	<5min	Incomplete, but damaged †
Vaporized hydrogen peroxide (VHP) ‡	Expose to 1.07 mg/L of VHP under conditions of 400 Pa, 22.8 ºC, <13.9%RH	Incomplete (treated for 9 h)	<0.5 h	<0.5 h	Incomplete (treated for 9 h)
Plasma	Oxygen-plasma	Incomplete (treated for 8 h)	ca. 8 h	ND	<2h
	Argon-plasma	4 h	Incomplete (treated for 8 h)	ND	4h

*) Processing temperature at surface of coupons were 122.0±0.3ºC and 141.9±0.5ºC treated with 120ºC- and 140ºC-oven, respectively.
†) When the samples were left over 60min, 7 Log and 8 Log reduction were observed in spores of *B. atrophaeus* and *A. niger*, respectively.
‡) VHP was supplied and discharged continuously at 0.932 mg/L/min by vacuuming.

the other strains. In contrast, it did not show resistance to UV light and argon plasma. *D. radiodurans* showed higher resistance to UV light and argon plasma than *B. atrophaeus* but did not show resistance to other treatment, except for oxygen plasma treatment. *A. niger* were resistant to UV light, similar to that of *D. radiodurans*, and also showed resistance to 7.5% H_2O_2 and VHP, like *B. atrophaeus*. *B. diminuta* did not show resistance to all bioburden reduction methods used in this study.

Spacecraft decontamination processes can be divided into initial bioburden reduction before delivery to assembly facilities and additional bioburden reduction during assembly and testing. For initial overall bioburden reduction of spacecraft component before assembly, dry heat may be suitable if the material is heat resistant because dry heat can sterilize various microbial species, reproduce results well, and effect the surface and deep within the components. Methods other than dry heat should be carefully used for non-heat-resistant components and additional bioburden reduction in the cleanrooms. Chemical treatments are more effective when applied with wiping than when used alone. In addition to the low-pressure plasma tested in this study, portable atmospheric-pressure plasma may be effective in additional bioburden reduction. The anticipated flow of the spacecraft components from the introduction into the facility to assembly and launch is shown in Figure. 1, and bioburden monitoring at each of these stages is essential.

This study quantitatively compared the effectiveness of bioburden reduction techniques on typical resistant microbes. Future research can include microorganism detection using methods other than colony counting, expanding the range of microorganisms and sterilization methods, and investigating material compatibility. These studies help reduce spacecraft bioburden; however, they must be considered in conjunction with the type of exploration mission.

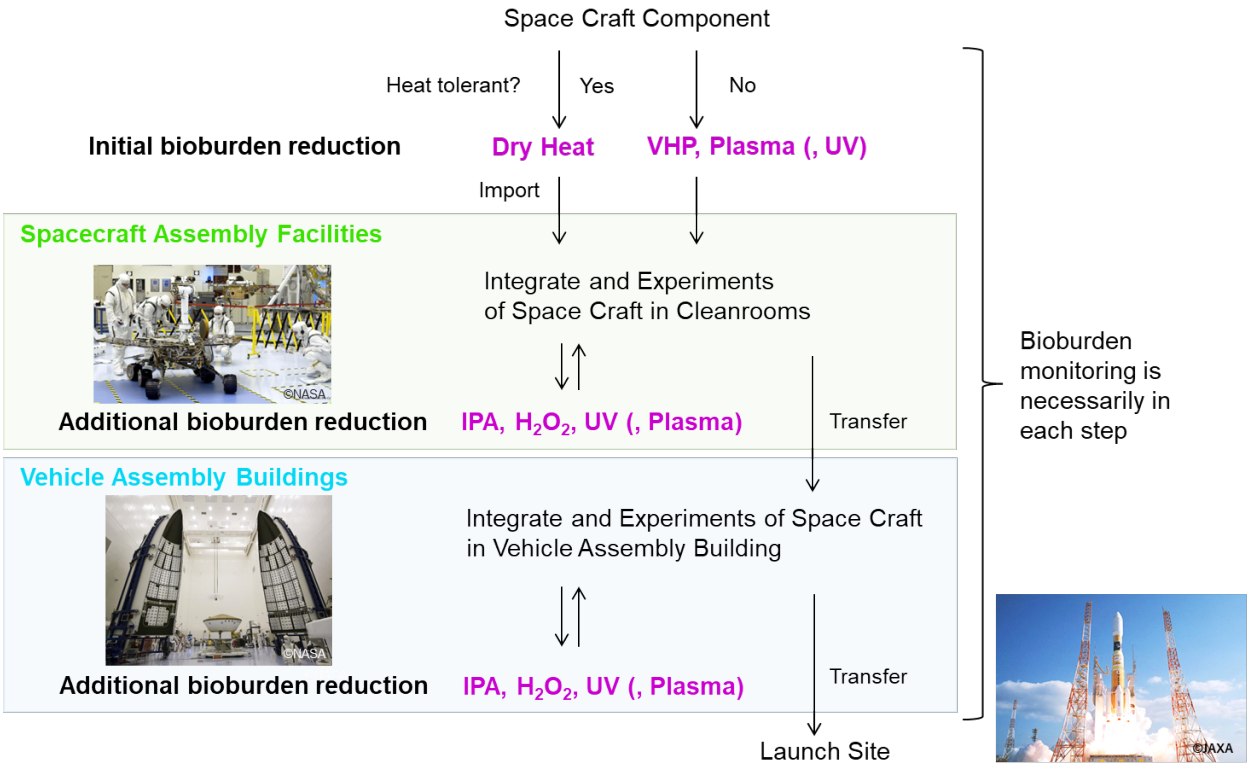

Figure 1. Conceivable bioburden reduction methods in the flow to integrate space craft (Revised from Kimura et al., under review).

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References

1) Kminek, G., Fellous, J. L., Rettberg, P., Moissl-Eichinger, C., Sephton, M. A., and Royle, S. H., et al. (2019). The international planetary protection handbook. An online-only supplement to Space Research Today, volume 205.
2) COSPAR (2021). COSPAR policy on planetary protection. https://cosparhq.cnes.fr/assets/uploads/2021/07/PPPolicy_2021_3-June.pdf [Accessed July 5, 2023].
3) Barengoltz, J. and Stabekis, P. D. (1983). U.S. planetary protection program: Implementation highlights. *Adv. Space Res.* 3, 5-12. doi: 10.1016/0273-1177(83)90166-7
4) European Cooperation for Space Standardization (2013). Space product assurance: Dry heat bioburden reduction for flight hardware, ECSS-Q-ST-70-57C.
5) European Cooperation for Space Standardization (2008). Materials and hardware compatibility tests for sterilization processes. ECSS-Q-ST-70-53C.
6) Patel, M., Gow, J., Paton, S., and Truscott, P. (2017). SterLim: Sterilisation limits for sample return planetary protection measures. ESA contract no. 4000112742/14/NL/HB
7) Craven, E., Winters, M., Smith, A.L., Lalime, E., Mancinelli, R., Shirey, B., et al. (2021). Biological safety in the context of backward planetary protection and Mars sample return: conclusions from the sterilization working group. *Int. J. Astrobiol*. 20, 1-28. doi: 10.1017/S1473550420000397
8) NASA (2022). Implementing Planetary Protection Requirements for Space Flight. NASA-STD-8719.27.