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Planetary protection on international waters: An onboard protocol for capsule retrieval and biosafety control in sample return mission

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Abstract

Planetary protection has been recognized as one of the most important issues in sample return missions that may host certain living forms and biotic signatures in a returned sample. This paper proposes an initiative of sample capsule retrieval and onboard biosafety protocol in international waters for future biological and organic constituent missions to bring samples from possible habitable bodies in the solar system. We suggest the advantages of international waters being outside of national jurisdiction and active regions of human and traffic affairs on the condition that we accept the Outer Space Treaty. The scheme of onboard biological quarantine definitely reduces the potential risk of back-contamination of extraterrestrial materials to the Earth.

Keywords: Planetary protection; International waters; Onboard protocols; Ongoing chemical evolution; Unexplored biosphere

1. Introduction

From the time of the Apollo Project in the 1960–1970's, sample return missions from the solar system bodies have significantly contributed to advance researches on the origin and evolution of the solar system. In the future, further explorations to Mars and icy satellites such as Enceladus and Europa will be definitely settled as sample return missions (*e.g.*, McKay and Davis, 1989; DeVincenzi et al., 1998; National Research Council, 2012; Tsou et al., 2012; Sekine et al., 2014). Issues of planetary protection are generally handled by the space community, although these issues often come to the attention of various academic communities and the public (*e.g.*, Rummel, 2001; Rummel, 2002a; Rummel and Billings, 2004). Since the establishment of the Panel on Planetary Protection (PPP) and the Committee on Space Research (COSPAR), an international consensus has emerged regarding the development and promulgation of planetary protection knowledge and policies, and regarding plans for mitigating the harmful effects of biological contamination on Earth (*e.g.*, Rummel

Abbreviations: COSPAR, Committee on Space Research; PPP, Panel on Planetary Protection; R/V, research vessel; BSL, biosafety level; UNCLOS, United Nations Convention on the Law of the Sea; OST, Outer Space Treaty; WHO, World Health Organization

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et al., 2002b; Crawford, 2005; Nicholson et al., 2009). The investigation of biological quarantine for planetary protection against both forward- and back-contamination has been discussed from the viewpoint of risk management and public consensus, in the context of further planetary exploration (*e.g.*, Rummel, 2009; Adler et al., 2011; Moissl-Eichinger, 2011). Planetary protection and quarantine protocols has been updated including the strategy of sampling and sub-sampling for the detection of life signatures (Rummel et al., 2002c; Allwood et al., 2013). However, selection of a candidate location for initial quarantine, especially for materials with high biosafety levels (BSLs; *i.e.*, BSL 3 or 4), is problematic due to the potential risk of biological back-contamination and the difficulty of obtaining public consensus in the host countries of the sample recovery site.

To resolve key issues related to extraterrestrial sample return projects, we suggest that international waters (*i.e.*, areas of oceans, seas, and waters outside of national jurisdiction; Fig. 1) are a meaningful option for quarantine location on the condition that we accept the responsibility for the Article VI in the Outer Space Treaty (OST): "States Parties to the Treaty shall bear international responsibility for national activities in outer space, including the Moon and other celestial bodies, whether such activities are carried on by governmental agencies or by non-governmental entities, and for assuring that national activities are carried out in conformity with the provisions set forth in the present Treaty." To conduct an initial investigation of onboard biological quarantine, we propose application of a BSL-controlled laboratory on a developed research vessel for the operation of future sample return missions during the inbound planetary protection.

2. Onboard laboratory for unseen extreme biosphere

Marine environments, which play a significant role in global energy and material cycles and biogeochemical processes, and represent one of the largest unexplored biosphere and biomass reservoirs in this planet, including both water-column and sub-seafloor environments. Historically, the discovery of submarine hot springs in the Galapagos Lift, the East Pacific (Corliss et al., 1979) led many researchers and public peoples to consider that deep-sea hydrothermal systems are the most plausible places for chemical evolution of biomolecules, and for the subsequent origin and early evolution of present life in the Earth (*e.g.,* Holm, 1992; Gold, 1992). Deming and Baross (1993) introduced a concept that deep-sea smokers could be windows to the subsurface biosphere. Presumably, there are unexplored pristine prebiotic worlds beneath the biosphere, as indicated by numerous laboratory simulation and model studies (*e.g.,* Holm, 1992; McCollom and Seewald, 2007: cf. possible biological limitation factors, Rothschild and Mancinelli, 2001).

Further *in situ* dive expedition and drilling expeditions into the Earth's subsurface in the last decade have revealed that ecosystems consisting of diverse communities of prokaryotic cells occur deep in sub-seafloor crusts and sediment (*e.g.*, D'Hondt et al., 2004; DeLong, 2004; Kelley et al., 2005; Devey et al., 2007; Martin et al., 2008; Fry et al., 2008; Edwards et al., 2011; Hoehler and Jørgensen, 2013), maximally at depths of down to 1.6 km below the seafloor (Roussel et al., 2008) in the limited bioavailable carbon budget (Kallmeyer et al., 2012; Hinrichs and Inagaki, 2012). The deep biosphere that includes microbial communities in both the deep-sea and deep subseafloor environments is placed at "boundaries between habitability

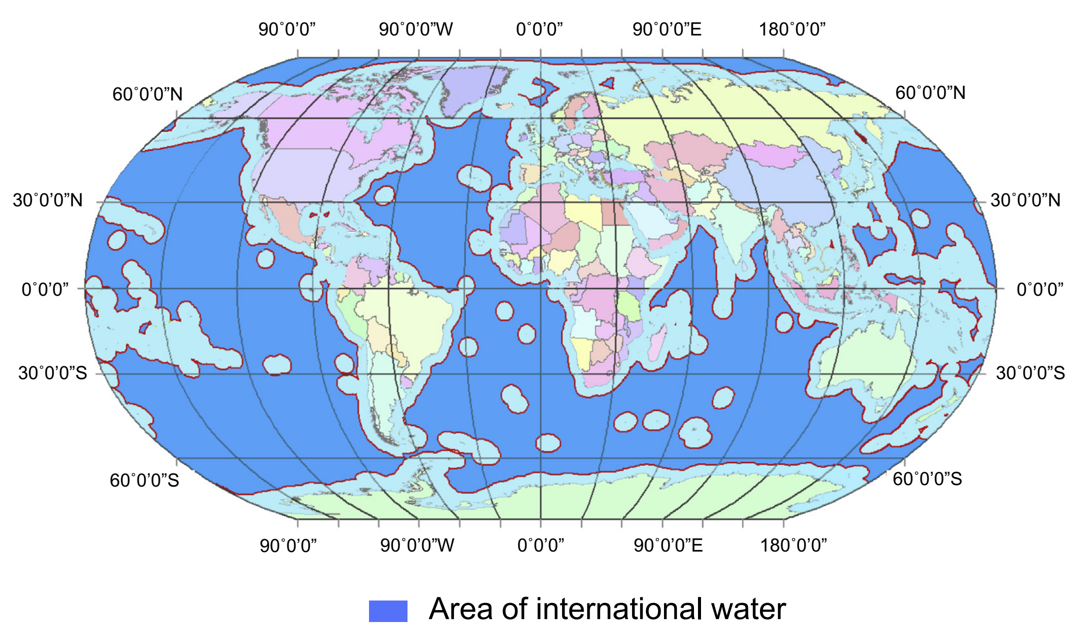

Fig. 1. Global distribution of international waters (dark blue; data of geographic information is referred from marineregions.org.).

and uninhabitability" in this planet in the view of mechanical, physical and chemical constraints of living forms such as cell space, gas–water availability, temperature, pressure, and many energy and elemental resources for life (cf. Holm, 1992; Holm and Charlou, 2001; Nealson et al., 2005; Hoehler, 2007; McCollom and Seewald, 2007; Proskurowski et al., 2008; Takai, 2012). In addition, many researches have pointed out that the microbial components in the deep biosphere are highly different from those in the surface world in the compositional, functional, physiological, and evolutionary contexts (*e.g.*, Jørgensen and Boetius, 2007; Lipp et al., 2008; Teske and Sorensen, 2008; Schrenk et al., 2010; Takano et al., 2010; Biddle et al., 2012; Lomstein et al., 2012; Lloyd et al., 2013). Thus, the exploration of the deep biosphere is an opportunity for encounter of unknown microbial activities in this planet.

The deep-sea research vessel *Chikyu* (*e.g.*, Curewitz and Taira, 2006) is a main platform of the International Ocean Discovery Program (IODP) that expands the frontiers of science, technology, and international collaboration (cf. Initial Science Plan of the IODP; http://www.iodp.org). The *Chikyu* (length, 210 m; gross tonnage, 56,752 tons; Fig. 2) is outfitted with comprehensive and advanced scientific research facilities (Table 1). One of the purposes of the vessel is to investigate the unexplored deep biosphere in oceanic regions of the Earth, including unseen extremophiles and microbial habitats (*e.g.*, D'Hondt et al., 2007). The *Chikyu* onboard laboratories offer physically contained systematic chemical and microbiological protocols (Fig. 3). Further development of new methodologies and technologies in the *Chikyu* has been directly coupled with new findings of the subseafloor microbial communities and their ongoing metabolic activities.

Table 1
Profile of the research and drilling vessel *Chikyu*. Experiments and handlings of BSL 2 or higher categories of microorganisms are not currently permitted on the *R/V Chikyu* (Chikyu biosafety guideline, 2011).

Main dimensions	
Length overall	210 m
Breadth overall	38 m
Depth	16.2 m
Height (sea-level)	112 m
Operational draft	9.2 m
Gross Tonnage	56,752 ton
Total crew/accommodation	Cabins for 200 persons
Cruising speed	11.45 knots with 5 thrusters
Helicopter deck	Capable for EH101
Power equipment	
Main generator for 6 sets	5000 KW
Aux. generator for 2 sets	2500 KW
Emergency generator for 1 set	560 KW

3. Biological quarantine on international waters: a proposal

According to the United Nations Convention on the Law of the Sea (UNCLOS), international waters are defined as all waters beyond national boundaries with freedom of navigation and also freedom of scientific research (*see*, Article 87: Freedom of the High Seas). We expect that the international waters are the most likely place for the future public consensus of onboard biosafety protocols because of the most rapid and convincing processing of the subsequent scientific

Fig. 2. Research vessel (*R/V*) *Chikyu*. Other research vessels (*e.g.*, *R/V Yokosuka* and *Natsushima* in JAMSTEC) are also capable of supporting the research operations and activities and the logistic tasks associated with many of the pre-, co- and post- expeditions by the *Chikyu*. Furthermore, the onboard environment can provide sufficient space for accommodation of working research and technical staffs (Supplementary information).

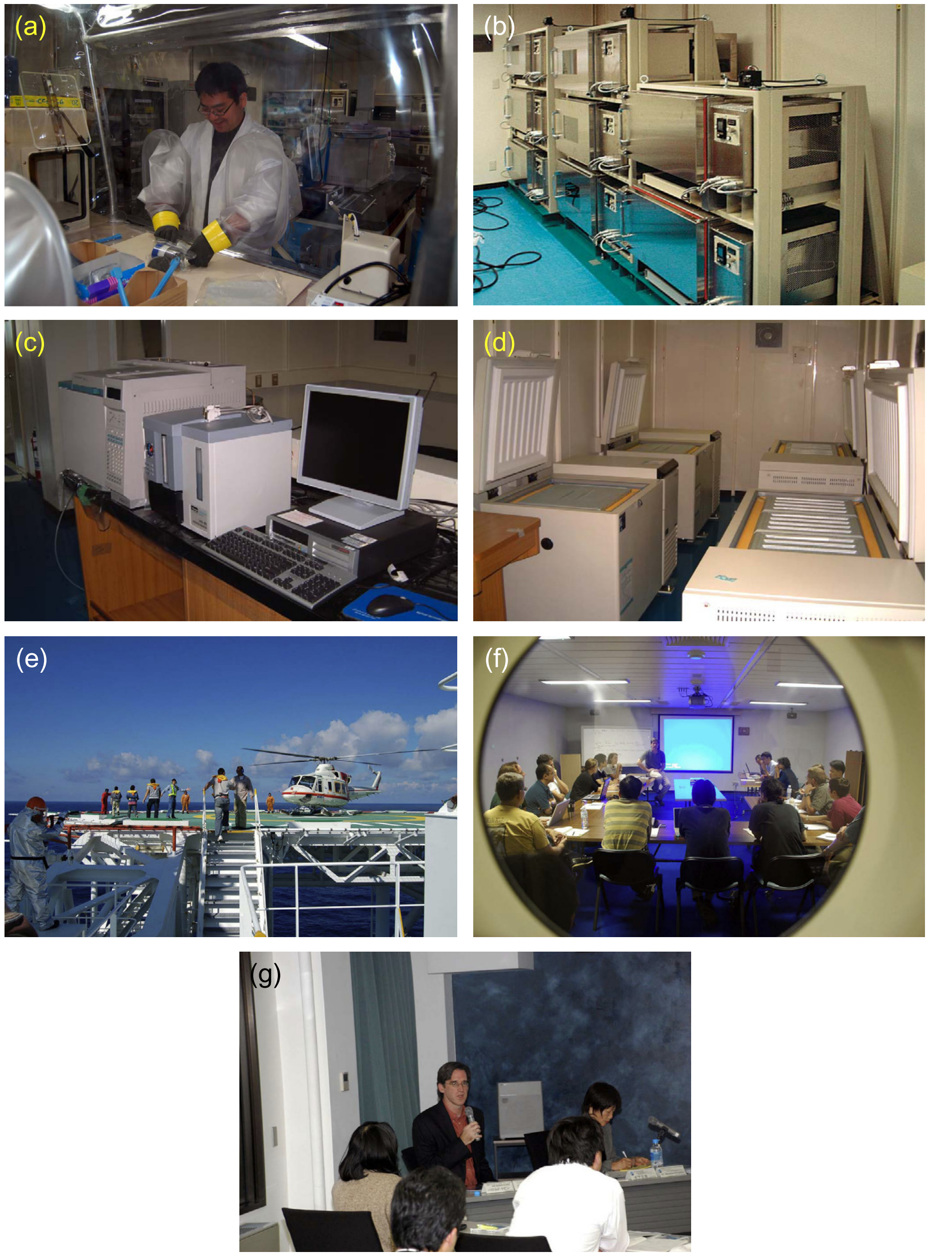

Fig. 3. Some overview of laboratory facilities on the *Chikyu*. (a) Anaerobic glove box in the microbiology lab, to prevent alteration or contamination of samples, (b) thermally controlled incubation systems in the microbiology lab, (c) gas chromatograph for analyzing gasses and liquids in the chemistry lab, (d) deep freezer systems for microbial samples maintained at −80 °C to −150 °C, (e) deck for helicopter operations, (f) meeting space for onboard scientific party, and (g) onboard press center for broadcasting.

research by well-disciplined specialists. On this basis, we propose potential onboard protocols for the sample recovery and the initial chemical and biological controls in future extraterrestrial sample return missions.

Development of the onboard laboratory to high BSL standards, based on the guideline of World Health Organization (WHO) (2004), will enable the sample and specimen handlings under physical containment. In addition, the planetary protection protocol will require sophisticated equipment which applicable to small scale analysis. Currently, for example, *Chikyu* has a sophisticated instrument including 3-dimensional X-ray Computed Tomography Scanner (3D X-ray CT), Gas Chromatograph combined with several types of detector (*e.g.*, flame ionization detector, thermal conductivity detector), High Performance Liquid Chromatography (HPLC), Fluorescence Microscope, Inductively Coupled Plasma Atomic Emission Spectroscopy (*i.e.*, ICP-AES for major cation, silicate, major elements), Inductively Coupled Plasma Mass Spectrometry (*i.e.*, ICP-MS for trace cation, silicate, trace elements), Laser Ablation ICP-MS for spot analysis and Scanning Electron Microscope/Energy Dispersive Spectroscopy (SEM–EDS) (please see also, Supplementary information). Table 2 represents a summary of the onboard quarantine scheme from scientific, political,

Table 2
Possible advantages of onboard quarantine and its comparison with onshore schemes, from (a) scientific, (b) political, and logistical perspectives. A helicopter deck and other research vessels can also provide logistical support using safe transportation protocols (Supplementary information). See the guidelines of the World Health Organization (2004) and practical reviews in the literature (*e.g.*, Crane et al., 1999; Le Duc et al., 2008).

	(a) Scientific aspects		
	Contamination risk	Requirement	Other remarks
Onboard quarantine	By water and air	WHO's approval for high BSL system (e.g., *Chikyu*)	Applicable platform for other sample return opportunities
Onshore quarantine	By soil, sand, water and air	Host BSL facility dependent	Need independent consensus/ scheme for other sample return opportunities
	(b) Political and logistical aspects		
	Candidate location	Public consensus	Access to landing site
Onboard quarantine	International waters (no nationality)	Obtainable (cf. PPP's approval)	Applicable anywhere on water with helicopter operation (e.g., *Chikyu*)
Onshore quarantine	Anökumene (e.g., desert)	Obtainable (host country dependent)	Applicable but limited situation

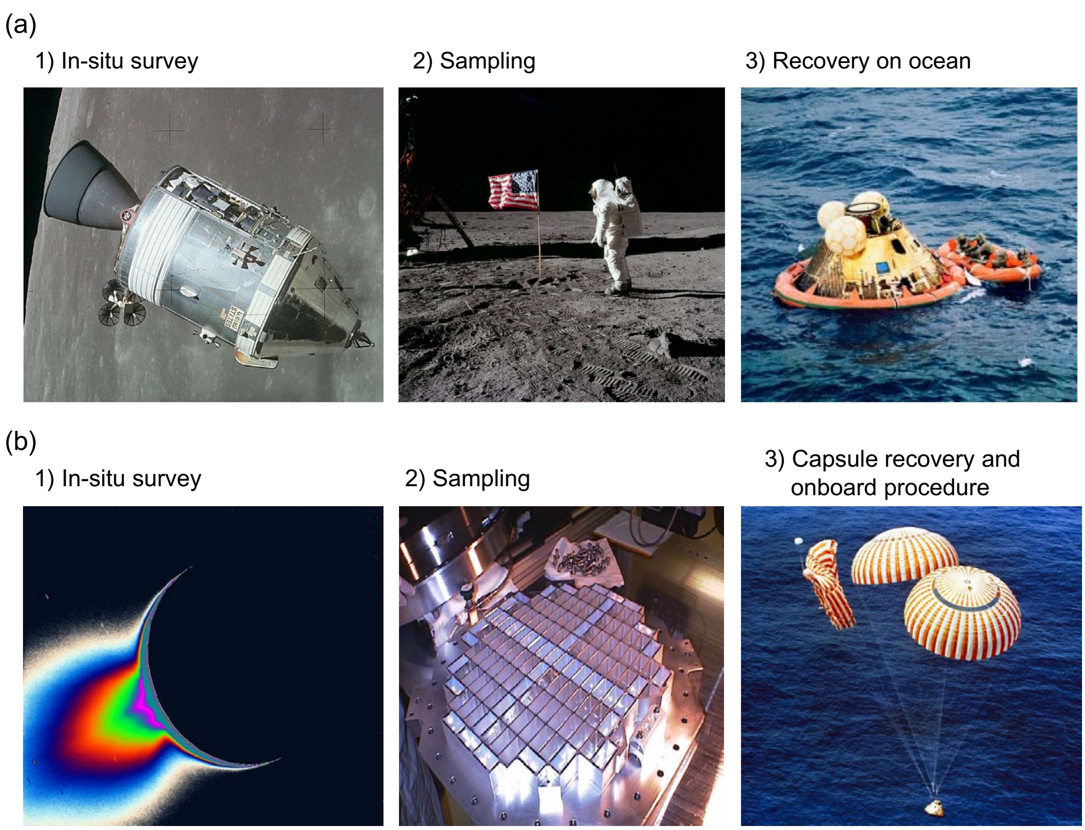

Fig. 4. Comparison of the capsule retrieval scheme on surface ocean during (a) Apollo mission (actually, Apollo-11 was returned on 24-July-1969; photo courtesy from great images in NASA), and (b) our proposal discussing in this paper with jet plume of sea water from Enceladus (Porco et al., 2006). Please see the context of aerogel trap dust collector to capture hypervelocity particles (Tsou et al., 2012). The photo of the returned capsule in Apollo mission-15 is courtesy from NASA.

and logistical perspectives, and a comparison with onshore-based quarantine schemes. Using the onboard protocols, initial sample handling will be performed by the restricted and trained scientists in the physically controlled laboratory environments (e.g., Le Duc et al., 2008). After declaration of safety statement for the returned sample, the sample can transit to onshore laboratories for further sample analysis, as required.

Alternatively, the returned sample can be transferred to onshore higher BSL facilities, if necessary. Currently, 26 locations of BSL 4 facilities operate in 15 countries including Australia, Canada, the Czech Republic, France, Gabon, Germany, India, Italy, Russia, South Africa, Sweden, Switzerland, Taiwan, the United Kingdom, and the United States. The facilities in Japan, *i.e.*, the National Institute for Infectious Diseases (Tokyo, Japan) and the Institute of Physical and Chemical Research (Tsukuba, Japan), have BSL 4 operating facilities (Kurane, 2009).

4. Application of the onboard protocol

Detailed analyses for the samples returned from the solar system objects, such as the Moon and small bodies, have greatly advanced our understanding on the origin and evolution of Earth and the solar system (*e.g.*, Wood et al., 1970; Flynn et al., 2006; Tsuchiyama et al., 2011). In response to recent findings of geological and cosmochemical activities on the solar system bodies (*e.g.*, McKay et al., 2008; Ehlmann et al., 2011; Schmidt et al., 2011; Turtle et al., 2011; Postberg et al., 2011), challenging sample return missions from these planets and satellites have been considered in addition to remote sensing and landing missions. For instance, the series of three Flagship missions to Mars, including the return of Martian rock samples to the Earth, was the highest priority of the Flagship mission recommended by the 2011 NASA's Planetary Decadal Survey. Additionally, a sample return mission concept from Enceladus' water-rich plumes (*i.e.*, LIFE mission; Life Investigation for Enceladus) is proposed to search extraterrestrial life in its interior ocean within a cost cap of Discovery or New Frontier program (cf. Tsou et al., 2012). These suggest that biological sample return missions will be able to be considered and made in the decades ahead in various mission sizes (*i.e.*, small, medium, and large), which in turn means that making concrete protocols for recovery and curation is necessary for preparing the coming decades of biological sample return missions (cf. ESR mission; Enceladus Sample Return from Sekine et al., 2014). Our proposal of onboard protocols in international waters would provide a concrete answer to address the issues of planetary protection of back-contamination in missions designed to return samples from planets and satellites potentially supporting life.

To date, capsule recoveries from outer space (circumsolar) trajectories are only achieved by in-land operation (*i.e.*, Genesis, Stardust and Hayabusa) while many capsule recoveries for Earth-bound trajectories have been achieved by both in-land and ocean operations (*e.g.*, Soyuz for in-land and Apollo for ocean, as shown in Fig. 4). One of important concepts in a sampler capsule design is capability of buoyant, likewise the returned capsule in the Apollo missions. Consequently, it should be noted that interplanetary capsule returns to international waters are still a new challenge for both Japan and the rest of the world. We need to establish careful contingency plans for capsule retrieval in case of off nominal range of trajectory correction maneuvers as well as prevention of drag/loss undersea by increasing the capsule durability, without severely contaminating returned samples for scientific purposes nor out-breaking them before safety declaration of planetary protection inspection.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.asr.2013.12.041.

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