METHOD FOR BIOLOGICAL CONTAMINATION MONITORING DURING AEROGEL CUTTING PROCESS IN TANPOPO PROJECT USING BIOLUMINESCENT BACTERIA *Photobacterium kishitanii*.

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INTRODUCTION

The Tanpopo mission is a Japanese astrobiological experiment which will be conducted on the Japanese Experiment Module (JEM) of the International Space Station (ISS) [1]. One of the goals of the Tanpopo mission is to capture microbes in space, possibly attached on the surfaces of micrometeoroids or space debris at the ISS orbit (approximately 400 km altitude). For this purpose, an excellent but fragile media called "silica aerogel" tiles will be used [2]. After the experiment, aerogel samples that possibly contain tracks with microbes/microbial DNA will be devided to regions using a device called "YOUKAN machine", and offered for PCR analysis. As PCR process amplifies any sort of microbial DNA, biological contamination of the returned sample in the curation laboratory must be strictly eliminated. To develop a suitable cutting method, biological contamination of returned aerogel during the proess should be monitored. In other words, sources (space or Earth) of microbes found in/on the returned aerogel sample should be clearly distinguished. In this report we focused on the measurement of microbes that should be dragged from the aerogel surface into the hole with the motion of the cutting needle. To measure only microbes from the aerogel surface (not those from the needle surface, etc.), here we report the use of bioluminescent bacteria, *Photobacterium kishitanii*.

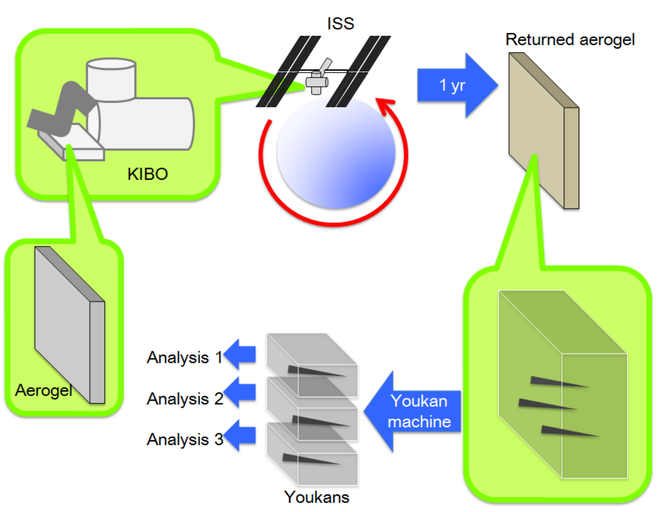

Fig. 1 A scematic illustration of the Tanpopo mission.

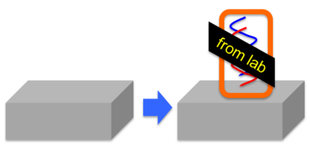

Fig. 4 An image of "laboratory originated" bacteria.

Fig. 5 *Photobacterium kishitanii* colonies on an agar plate.

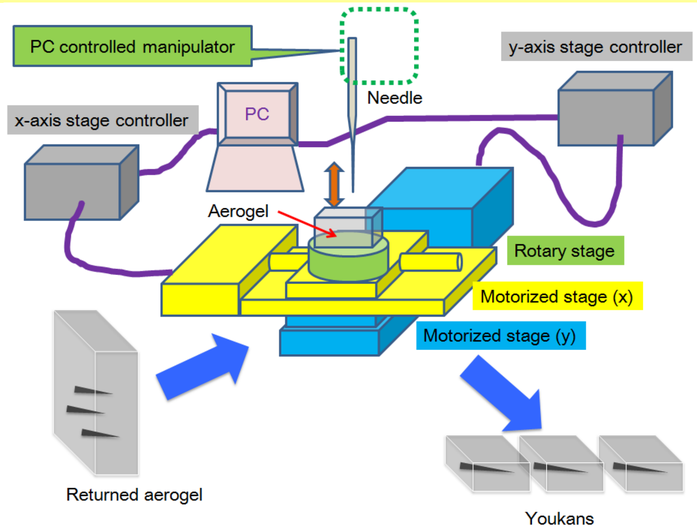

Fig. 2 Youkan machine. A returned aerogel will be separated into several Youkans and offered to analyses.

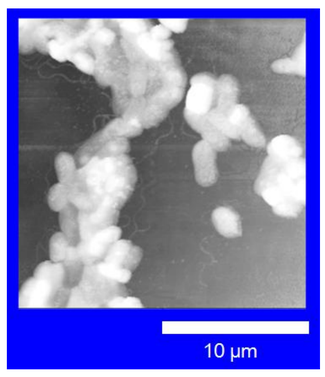

Fig. 6 An AFM image of *P. kishitanii*.

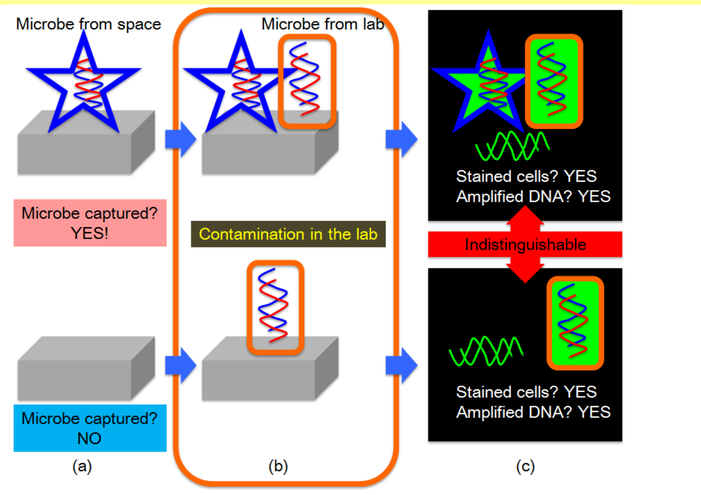

Fig. 3 A schematic illustration of microbial DNA detection procesure. Upper row represents the case with captured microbe, and lower without. Even with two different cases with and without space microbe captured in an aerogel (a), microbial contamination during the Youkan machine treatment (b) should result in the similar result when they are analysed using DNA staining dye (c).

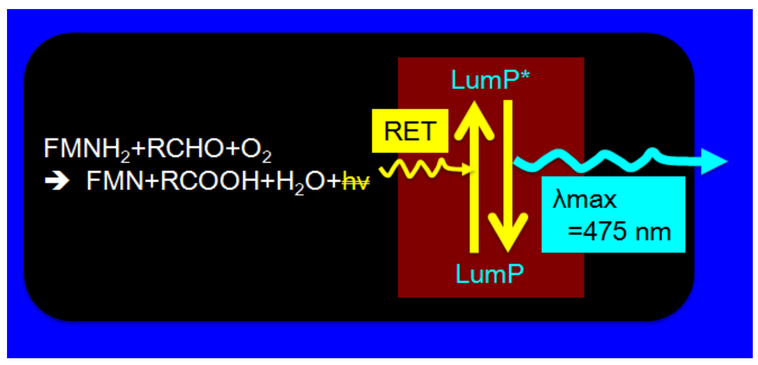

Fig. 7 Bioluminescence reaciton inside the bacteria.

BACKGROUND

In the TANPOPO mission, returned aerogels will be analyzed using various method by various researchers. Separation of the returned aerogel into scientifically significant pieces (Youkans) should be necessary for effective investigation (Fig. 1). The separation would be automatically performed using a system called "Youkan machine" which has recently been developed, similar to the one used in STARDUST mission by NASA [3], where the cutting needle vertically moved back and forth in z axis, together with the horizontal movement of aerogel fixed on x-y stage (Fig.2). Search for bacteria among the tracks of Youkans will be performed using PCR, with which possibly contained DNA fragments are amplified. Biological contamination of returned aerogel during the Youkan machine treatment must be, therefore, strictly eliminated, because microbes from space and from the lab are indistinguishable at the final DNA detection stage (Fig. 3). Even in the clean room a complete eliminaion of bateria is quite difficult beacuse of the existence of bacteria in the form of biofilm. It is quite important, terefore, to evaluate the bacterial contamination during the Youkan machine treatment. A model bacteria that can be labelled as "laboratory originated" is requred for the contamination estimation (Fig. 4). For this purpose, we focused on bioluminescent bacteria *Photobacterium kishitanii* . Among several bioluminescent bacteria, *P. kishitanii* emits strongest blue light with the peak wavelength of 475 nm (Fig. 5). This gram-negative cocci (Fig. 6) was collected from the skin of a cuttlefish for sashimi that was purchased at local supermarket. The blue light is emitted as a result of a chemical reaction that consumes oxygen and other substrates followed by an energy transfer to a color-regulating protein (Fig. 7). Methods for the attachment of this bacteria on the glass surface were studied, and continuous measurement of the luminescence were performed [4]. Measurement of luminescence from one single cell was also possible [5]. So far, no bioluminescent bacteria is found on land. This fact indicates that *P. kishitanii* would be a candidate for the model bacteria.

OBJECTIVE OF THIS STUDY

Characterization of aerogel-attached P. kishitanii bioluminescence and the investigation of its usability as a model bacteria for the contamination monitoring.

EXPERIMENT

Contamination measurement was performed in a procesure described in Fig. 8. Dried *P. kishitanii* was prepared by drying the bacterial colony at room temperature. The bacterial powder was attached on the wet needle surface. Time course of luminescence from the needle was performed by soaking the bacteria needle into marine broth (Fig. 9 (a)). All the measurements were performed at room temperature (25ºC).

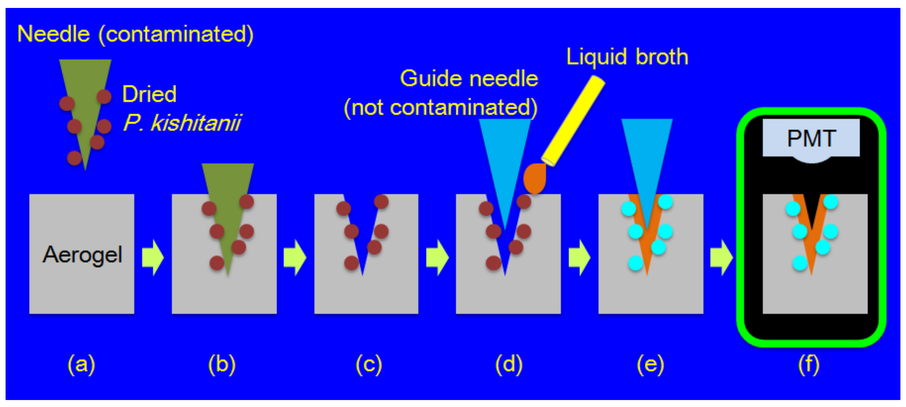

Fig. 8 Experimental procesure. Firstly, dried *P. kishitanii* cells were attached on the needle surface (a). The needle was carefully inserted into an aerogel (0.01 g cm^{-3}) (b). After the removal of the needle (c), a guide needle was placed into the hole (d) and 2-3 μL of liquid broth (marine broth) was added (e). Luminescence intensity from the P. kishitanii attached aerogel was measured using a luminometer (Lumicounter 200; Microtech Co., Funabashi, Japan) (f).

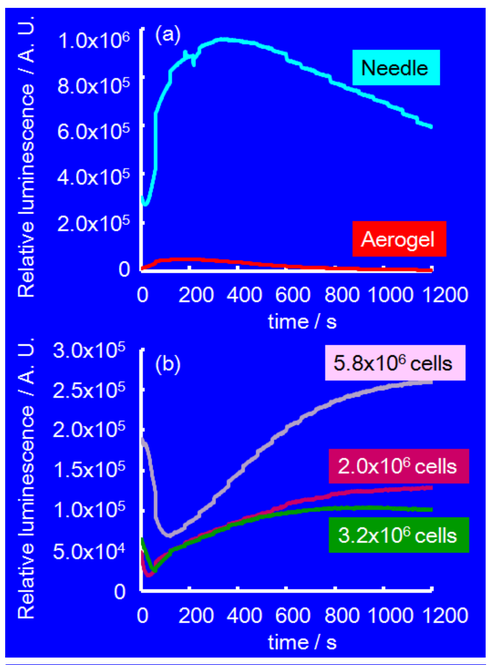

Fig. 9 Time courses of luminescence from *P. kishitanii* attached needle and aerogel (a). Those of dried cells in test tubes (b).

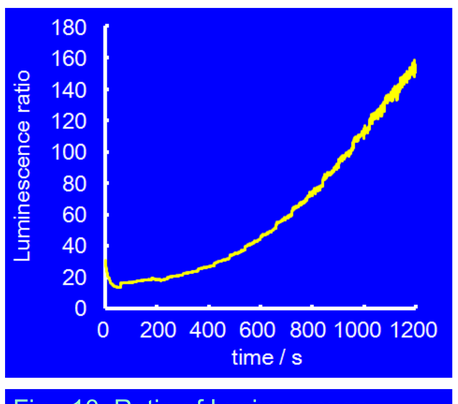

Fig. 10 Ratio of luminescence intensities from *P. kishitanii* attached needle to that from aerogel (time course).

RESULTS AND DISCUSSION

Both the luminescence curves in Fig. 9 (a) showed peaks at ca. 200-300 s. After the supply of liquid broth, the cells might had reactivated the luminescence reaction by consuming the fuel substrates that they had, until they are exhausted. After 400 s the cells had no available fuel for luminescence and the luminescence intensity dropped. A rough estimation of the aerogel-attached bacterial cells could be perfprmed using the result in Fig. 10. The curve was prepared as the ratio of needle luminescence to the aerogel luminescence in FIg. 9 (a). At around 200 s the ratio is stable at the value of 20. Considering the doubling time of the bacteria to be ca 60 – 90 min, cell division effect on the luminescence shoule be very small, and the ratio should be initially depended on the cell number ratio. The value 20 represented, therefore, the cell number ratio on two places. Dried cells in test tubes, on the other hand, showed increased luminescent intensity after 50-100 s. Difference between the curves in Fig. 9 (a) and (b) were, presumably, due to the attached environment of the bacterial cells, as the luminescence/growth characteristics were reported to be diffeerent in attached and suspended state [4]. A method to fix calibration (relative luminescence ➔ cell number) is needed, by assimilating the luminescence reactivation environment of aerogel-attached bacteria.

CONCLUSION

Bioluminescent bacteria should provide an effective, contactless tool to estimate bacterial contamination during the Youkan separation procedure.

References: [1] Yamagishi A. et al. 2009. Trans. JSASS Space Tech. Jpn. 7: Tk 49-Tk 55. [2] Tabata M. et al. 2011. Biol. Sci. Space. 25: 7-12. [3] Westphal A. J. et al. 2004. Meteorit. Planet. Sci., 39: 1375-1386. [4] Sasaki S. et al. 2012. Chem. Lett. 41: 1213-1214. [5] Sasaki S. et al. 2009. Lett. Appl. Microbiol. 48:313-7.