Complete Life Cycle and Heat Tolerance of Dry Colonies of a Terrestrial Cyanobacterium, *Nostoc* sp. HK-01

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Abstract

The exact life cycle of cells in a terrestrial cyanobacterium, *Nostoc* sp. HK-01 which has several different types of cells, was confirmed by microscopic observation. All of types of cells were individually observed during 30-days of liquid incubation. The germination process from akinetes was recognized twice during the incubation period. The contribution of extracellular polysaccharides, EPS, for tolerance to heat in the dried colonies of *Nostoc* sp. HK-01 was also investigated. The survival rate of dried colonies of *Nostoc* sp. HK-01 with and without EPS were examined after exposure to temperatures at 100°C. The contribution to heat tolerance of EPS was tested and verified by the cell staining method. The survival rate of both groups of cells, with EPS and without EPS, of *Nostoc* sp. HK-01 was not remarkably different and they both lived under these conditions for 12 hours. These results indicate that the contribution of EPS to their heat tolerance would be very low. The amounts of sucrose and trehalose in the dry colonies were investigated to determine the contribution to heat tolerance in akinete as a compatible solute. The amount of trehalose was lower than that of sucrose in the dry colonies. It can be assumed that trehalose did not contribute to their heat tolerance. ©2017 Jpn. Soc. Biol. Sci. Space; doi: 10.2187/bss.31.1

Keywords; compatible solute, cyanobacteria, extracellular polysaccharides, heat tolerance, life cycle of cell, *Nostoc* sp. HK-01

Introduction

The preparations for the establishment of a manned Mars mission have been starting and lots of results related to the mission have been accumulated (Badescu, 2009; Casaregola *et al.*, 2009; Ehrenfreund *et al.*, 2012; International Space Exploration Coordination Group, 2013; Salotti and Heidmann, 2014). As one of the aspects of a the mission, we have been focusing on the study of space agriculture for human habitation on Mars (Yamashita *et al.*, 2006, 2009; Wada, 2007; Kanazawa *et al.*, 2008). In the study of space agriculture, terrestrial cyanobacteria are the best candidates for initial organisms to introduce to Mars, because of their various abilities; photosynthesis, nitrogen fixation and drought tolerance (Ohmori *et al.*, 2006; Obana *et al.*, 2007; Yamashita *et al.*, 2009; Garcia-Pichel, 2010; Hu *et al.*, 2012; Katoh *et al.*, 2012).

There is a terrestrial cyanobacterium, *Nostoc* sp. HK-01, which was isolated and characterized from *Nostoc* commune crusts (Katoh *et al.*, 2003). Arai *et al.*, (2008) and Kimura *et al.* (2015b) found that *Nostoc* sp. HK-01 can grow for a period of 140 days, and also can survive for over eight years on Martian regolith simulant which does not include a carbon source or a nitrogen source. In addition, the cyanobacterium has high tolerances to several cosmic rays (Tomita-Yokotani *et al.*, 2013). *Nostoc* sp. HK-01 may be suitable to create organic soil from Martian regolith (Arai *et al.*, 2008; Arai, 2009; Oarga, 2009; Verseux *et al.*, 2016). The result indicates that the cyanobacterium can also be used to add nutrition to harsh environments on Earth. There are many harsh environmental places for aerobes where temperatures are around 60°C even on the Earth (Gao, 1998). Kimura *et al.* (2015a) has found that only the akinetes in dry colonies of *Nostoc* sp. HK-01 have the ability to tolerate to heat. The mechanisms, however, have not yet been elucidated.

Aside from tolerances to heat, it has been investigated that the cyanobacterium, *Nostoc* sp. HK-01, has tolerances to desiccation, NaCl and vacuum (Arai *et al.*, 2008; Yoshimura *et al.*, 2012). In the case of tolerances to desiccation, NaCl and ultraviolet (UV), the contribution of EPS, extracellular polysaccharides, was deeply related to each tolerance, e.g., the chemicals in the EPS have the abilities to protect cells from damage by ultraviolet rays (Ehling-Schulz *et al.*, 1997; Tamaru *et al.*, 2005; Knowles and Castenholz, 2008; Yoshimura *et al.*, 2012).

There are some reports that trehalose has an important role in the drought-resistance of *Polyoedihum vanderplanki*, *Tardigrades* and other organisms, as a compatible solute (Blomberg, 2000; Elbein *et al.*, 2003; Sakurai *et al.*, 2008; Welnicz *et al.*, 2011). Hershkovitz *et al.* (1991) reported that two kind of cyanobacteria; *Phormicium autumnale* and *Chroococcidiposis* sp., accumulated trehalose and sucrose during a period of drought. Then, we tried to analyze the substances, trehalose and sucrose, for their contribution to heat tolerances as compatible solutes in *Nostoc* sp. HK-01.

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Fig. 1. Cell types of *Nostoc* sp. HK-01 during an incubation period of 24 days ($\times 400$). Arrows (▲) show heterocyst and arrowheads (▲) show the young akinetes. Bar shows 20 μm .

In this study, the exact life cycle of cells in a terrestrial cyanobacterium, *Nostoc* sp. HK-01 was determined. And the contribution of extracellular polysaccharides, EPS, for tolerance to heat in the dried colonies of *Nostoc* sp. HK-01 was also investigated. Furthermore, we tried to investigate the amounts of trehalose and sucrose as candidate compatible solutes in the cells which have heat tolerance.

Material and Methods

Cyanobacterium

A pure culture of a terrestrial cyanobacterium, *Nostoc* sp. HK-01 (NIES-2109, Algae Resource Database), was used as material in all experiments in this study (Kimura *et al.*, 2015a).

Incubation and preparation of dried colonies

The preparation of dried colonies was conducted as described previously (Kimura *et al.*, 2015a). 2.1×10^6 *Nostoc* sp. HK-01 cells were incubated in the liquid medium, BG-11 (Rippka *et al.*, 1979), at 26°C, under the light condition, $74.3 \pm 24.3 \mu\text{mol m}^{-2} \text{s}^{-1}$, with shaking at 120 rpm (BW101, Yamato, Japan). After the incubation, the wet colonies were dried at 23°C with relative humidity around 30% in the dark. The colonies were naturally dried under those conditions until the weight was stable.

The preparation of cells without EPS and confirmation of cells without EPS

The preparation of cells without EPS was carried out according to the method of Tamaru *et al.* (2005) with modification. The dried colonies of *Nostoc* sp. HK-01 were hydrated with 25 mM HEPES-NaOH (pH 7.0) and stored overnight at 25°C. The hydrated colonies were concentrated to collect the cells at $2000 \times g$, 10 min, at 20°C after they had been washed with 25 mM HEPES-NaOH (pH 7.0) two times. Astra-blue, which can stain sugars, was used for the confirmation of cells without EPS compared to the cells with EPS as a control (Tamaru *et al.*, 2005; Hosonuma *et al.*, 2009). After the removal of EPS, cells were re-dried at room temperature under dark condition, over 2 weeks. The control group was hydrated with BG-11 and stored overnight at 25°C, and re-dried at room temperature under dark condition, over 2 weeks. Both dry colonies with and without EPS, ca. 100 μg dry weight, were placed in an oven (WFO-450, Eyela, Japan), and exposed to 100°C for 1 to 12 h.

Pre-incubation for each survival experiment

Dry and heated colonies were rehydrated by 500 μL of BG-11 liquid medium. Each colony was kept in the dark at $30 \pm 2^\circ\text{C}$ for 2 days and stirred for 1 minute by a mixer (TM-251, Iwaki, Japan), and used for the viability assay.

Table 1. Relative numbers of each cell type of *Nostoc* sp. HK-01 the during incubation period. Each value means average $\pm$ standard error (n = 6). The graph is shown in Fig. 2.

Incubation period (Day)	Cell types			
	Akinete	Vegetative Cell	Hormogonia	Heterocyst
0	100.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
1	90.4 $\pm$ 2.5	9.6 $\pm$ 2.5	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
2	29.6 $\pm$ 4.4	70.4 $\pm$ 4.4	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
3	4.9 $\pm$ 1.9	95.1 $\pm$ 1.9	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
4	2.8 $\pm$ 1.8	97.2 $\pm$ 1.8	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
5	1.0 $\pm$ 0.4	99.0 $\pm$ 0.4	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
6	0.4 $\pm$ 0.2	93.5 $\pm$ 3.9	6.1 $\pm$ 3.8	0.0 $\pm$ 0.0
7	0.6 $\pm$ 0.4	88.6 $\pm$ 6.3	10.7 $\pm$ 6.3	0.0 $\pm$ 0.0
8	0.6 $\pm$ 0.3	94.9 $\pm$ 0.5	0.0 $\pm$ 0.0	4.5 $\pm$ 0.5
9	0.0 $\pm$ 0.0	85.9 $\pm$ 3.6	10.3 $\pm$ 3.8	3.9 $\pm$ 0.6
10	0.0 $\pm$ 0.0	72.4 $\pm$ 7.7	24.3 $\pm$ 8.3	3.3 $\pm$ 0.9
11	0.0 $\pm$ 0.0	71.0 $\pm$ 3.1	25.5 $\pm$ 3.5	3.5 $\pm$ 0.9
12	0.0 $\pm$ 0.0	80.4 $\pm$ 2.4	14.0 $\pm$ 3.2	5.6 $\pm$ 1.4
13	0.0 $\pm$ 0.0	90.6 $\pm$ 1.4	0.5 $\pm$ 0.5	8.9 $\pm$ 1.4
14	0.1 $\pm$ 0.1	90.9 $\pm$ 1.4	1.6 $\pm$ 1.6	7.4 $\pm$ 0.5
15	0.5 $\pm$ 0.2	90.7 $\pm$ 1.1	0.0 $\pm$ 0.0	8.8 $\pm$ 1.0
16	0.1 $\pm$ 0.1	90.0 $\pm$ 1.0	0.8 $\pm$ 0.8	9.1 $\pm$ 0.7
17	0.6 $\pm$ 0.2	92.9 $\pm$ 0.8	0.0 $\pm$ 0.0	6.5 $\pm$ 0.9
18	0.7 $\pm$ 0.3	91.6 $\pm$ 0.8	0.4 $\pm$ 0.4	7.2 $\pm$ 0.4
19	1.4 $\pm$ 0.4	92.1 $\pm$ 0.9	0.2 $\pm$ 0.2	6.3 $\pm$ 0.6
20	1.3 $\pm$ 0.5	91.8 $\pm$ 2.0	2.1 $\pm$ 1.3	4.8 $\pm$ 0.4
21	2.8 $\pm$ 1.0	89.6 $\pm$ 1.3	1.4 $\pm$ 1.4	6.2 $\pm$ 0.3
22	2.3 $\pm$ 0.9	91.0 $\pm$ 0.9	1.3 $\pm$ 1.3	5.4 $\pm$ 0.5
23	1.5 $\pm$ 0.3	94.0 $\pm$ 0.8	0.0 $\pm$ 0.0	4.5 $\pm$ 0.6
24	1.5 $\pm$ 1.1	93.7 $\pm$ 1.7	0.6 $\pm$ 0.6	4.2 $\pm$ 0.6
25	4.2 $\pm$ 1.2	79.1 $\pm$ 5.0	12.3 $\pm$ 5.7	4.4 $\pm$ 0.5
26	1.7 $\pm$ 0.5	83.9 $\pm$ 6.3	8.0 $\pm$ 6.5	6.4 $\pm$ 1.0
27	3.7 $\pm$ 0.9	84.9 $\pm$ 2.0	5.3 $\pm$ 1.8	6.1 $\pm$ 0.7
28	3.0 $\pm$ 0.7	75.0 $\pm$ 4.9	17.4 $\pm$ 5.6	4.6 $\pm$ 0.5
29	3.2 $\pm$ 0.6	88.3 $\pm$ 1.7	3.1 $\pm$ 1.4	5.4 $\pm$ 0.5
30	2.7 $\pm$ 0.8	71.4 $\pm$ 14.8	23.0 $\pm$ 15.8	2.8 $\pm$ 0.8

The viability assay

The viability assay was performed as described previously (Kimura *et al.*, 2015a) using the FDA (Fluorescein diacetate) staining method (Jones and Senft, 1985; Mori *et al.*, 2002; Arai *et al.*, 2008). The viability assay was conducted at several stages to confirm the tolerance to heat.

At each tested stage, the 100 μ L FDA staining solution was added to the 200 μ L of suspended cells with water containing cyanobacteria, and mixed by shaking at 500 rpm, 37°C for 10 minutes in dark conditions (M-BR-022UP, Taitec, Japan). After 5 minutes on ice, the cells were observed using a fluorescent microscope (BX50, Olympus, Japan), with a fluorescence NIBA filter (excitation wavelength, 470-490nm). The viable cells emitted green fluorescence during observation.

The observation of growth and each morphological cell type

The differentiation of akinetes, vegetative cells, heterocysts and hormogonia were observed by microscope (BX50, Olympus, Japan). 100 trichome

cells were counted during the 0-7 day incubation period for calculation of growth. The number of observed individual types of cells was statistically counted on six random samplings of three 40 x 40 μ m compartments of photomicrographs during the 30-day incubation period.

Preparation of the colonies with different ratio of akinete cells

The method for incubation of *Nostoc* sp. HK-01 was the same as described above. After the incubation, two groups of incubated wet colonies; one with a low number of akinete cells, the other with a high number of akinete cells, were obtained by counting all of cells. The number of all the cells and the number of akinete cells were counted from the photomicrographs, using Thoma's counting chamber (Nitirin). Groups of around five percent of akinete, and around 60% of akinete were prepared. The akinete content ratio (%) was calculated as the number of akinete / the number of all cells $\times$ 100.

The amounts of trehalose and sucrose

The colonies with each ratio of akinete were extracted with 80% ethanol at 65°C. The solutions were evaporated

Fig. 2. Growth and each morphological cell type of *Nostoc* sp. HK-01. A: Growth of *Nostoc* sp. HK-01 during an incubation period of 7 days. B-1: Akinetes. B-2: Vegetative cells. B-3: Hormogonia. B-4: Heterocysts. C: Relative number of each cell types during incubation period of 30 days. ■: Akinetes. □: Vegetative cells. ▨: Hormogonia. ▩: Heterocysts. The values are shown in Table 1.

to dryness in vacuo at 38°C. The fraction was purified using a C₁₈ Sep-pak cartridge (Waters, USA) with a water-methanol solvent system by increasing the methanol concentration in the series of 0, 50, 100% (10 mL per step). The 0% methanol fraction was further purified through a 0.45 µm filter (Tosoh, Japan) and analyzed by LC-ESI⁺/MS. Each extract was analyzed by LC-ESI⁺/MS. The positive mode of LC-ESI/MS spectra and several fragments were analyzed with a Waters system (Waters micromass ZQ) consisting of a MS, (2690 Separation Module with a 996 Photodiode Array and a HPLC / PD detector) under the following operating conditions: capillary voltage, 3.5 kV; cone voltage, 30V; extractor voltage, 5V; lens voltage, 0.1 V RF; source temperature, 120°C; desolvation gas flow, 300L hr⁻¹; cone gas flow, 50.0 L hr⁻¹; column, TSKgel Amide80, Particle size: 3 µm, Dimension: 2.0 mm, I.D.×15 cm, Tosoh; column Temp., 30°C; solvent, CH₃CN (80/20); flowrate, 0.2 mL min⁻¹.

Results and discussion

To confirm the exact cellular life cycle of *Nostoc* sp. HK-01, all of types of cells in colonies of the strain during

their life cycle were observed during a 30-day incubation period. Fig. 1 showed all types of the observed cells of *Nostoc* sp. HK-01 during a 24-day incubation period. There are only akinetes at the initial state, and a similar picture was obtained after 1-day of incubation (Fig. 1-a and b). The vegetative cells were not recognized until after 2-days of incubation and elongated vegetative cells were observed after 4-days of incubation (Fig. 1-c and d). After around 6-days of incubation, hormogonia were observed (Fig. 1-e and f). After the occurrence of hormogonia, heterocysts were recognized after 8-days of incubation (Fig. 1-g). Then, the young akinetes were differentiated among the elongated vegetative cells (Fig. 1-h and i). After that new matured akinetes (Fig. 1-j and k) were re-germinated, and the new vegetative cells were elongated again (Fig. 1-l and m). Fig. 2A shows the growth of *Nostoc* sp. HK-01. 2.1×10^6 cells became 1.2×10^8 cells in 7 days of incubation. After 8 days of incubation, the number of trichome could not be counted because the division of trichome cells coincide with the differentiation of heterocyst or akinete cells. Annual changes of the number of each cell; akinetes (Fig. 2B-1), vegetative cells (Fig. 2B-2), hormogonia (Fig. 2B-3) and

Fig. 3. The life cycle of cells of *Nostoc* sp. HK-01. Arrow (▲) shows heterocyst. Arrowhead (▲) shows young akinete.

heterocysts (Fig. 2B-4), were counted (Fig. 2C and Table 1). The cell types and time course of the number of all types of cells are shown in Fig. 2C. Kimura *et al.* (2015a) have already reported that only akinetes of *Nostoc* sp. HK-01 can live after they have been dried. As shown in Fig. 2C, the relative number of akinetes was remarkably decreased around 4-days of incubation and the relative number of vegetative cells was increased along with a decrease in the number of akinetes, which indicates that some vegetative cells were germinated from akinetes. During the observation, some of the vegetative cells differentiated to hormogonia and heterocysts. Some akinetes developed among the vegetative cells (Fig. 1, Fig. 2 and Table 1). The existence of hormogonia was recognized after 10 to 11 days of incubation and the percentage of hormogonia was around 20 %, but the ratio of hormogonia was reduced to around one percent at day 13 and 14 of incubation. These results indicate that the hormogonia differentiated to be vegetative cells and furthermore the vegetative cells grew. The existence of heterocyst, which is a dead-end cell, has been recognized around 3 to 9 % after 8-days of incubation, which was observed in *Nostoc* sp. HK-01. These phenomena agree with several other reports (Adams and Duggan, 1999; Dworkin, 2006; Mateo *et al.*, 2011).

The life cycle of cells in *Nostoc* sp. HK-01 was summarized as shown in Fig. 3. The confirmation of a detailed life cell cycle was first revealed in *Nostoc* sp. HK-01. The revealed cell life cycle of *Nostoc* sp. HK-01 is far different from that of *Nostoc commune* although *Nostoc* sp. HK-01 was isolated from the colony of *Nostoc commune* (Katoh *et al.*, 2003). According to other researchers' lengthy studies, *Nostoc commune* does not have the ability of differentiation from their vegetative cells to akinetes (Cameron, 1962; Potts, 2000; Tamaru *et al.*, 2005). Tamaru *et al.* (2005) have reported that the after the removal of EPS from the colonies of *Nostoc commune*, they did not even show a strong tolerance

Fig. 4. Microscopic observation of *Nostoc* sp. HK-01 after the astra-blue staining (x 400). A: cells with EPS, B: cells without EPS. Bar shows 20 μ m.

to desiccation by air. Those results indicate that the character of *Nostoc* sp. HK-01 is far different from that of *Nostoc commune*.

The heat tolerance of *Nostoc* sp. HK-01 has already been reported but the contribution of extracellular polysaccharides, EPS, to tolerance to heat in the dried colonies has not yet been investigated. The preparation of cells without EPS was modified from the method of Tamaru *et al.* (2005). Fig. 4 showed the microscopic photographs of cells after the stain with astra-blue which can stain the sugars such as EPS. As shown in Fig. 4-B, treated cells were not stained with astra-blue. Thus, the method to remove EPS from the cells, used here, was proved to be useful in the case of *Nostoc* sp. HK-01.

The survival rates in dried colonies of *Nostoc* sp. HK-01 with and without EPS after exposure to 100°C were shown in Fig. 5 and Table 2. The survival rates of both groups of cells at the time of exposure, with EPS and without EPS, of *Nostoc* sp. HK-01 were not remarkably different and the cells lived under exposure to heat for 0 to 12 hours ($p \geq 0.05$) (Fig. 5 and Table 2). These results indicate that there are some functions in the cytoplasm of cells for tolerance to heat, which are different from that of the tolerance to UV, NaCl or desiccation in *Nostoc commune*, *Chroococcidiopsis* sp. CCME 5056,

Fig. 5. The survival rates of *Nostoc* sp. HK-01, cells with EPS and cells without EPS, after exposure to dry heat (100°C). The values are shown in Table 2.

Table 2. The survival rates of *Nostoc* sp. HK-01, cells with EPS and cells without EPS, after exposure to dry heat (100°C). The graph is shown in Fig. 5.

Heat exposure time (h)	Survival rate of cells (%)*	
	With EPS	Without EPS
0	44.9 ± 7.2	53.8 ± 4.9
2	30.6 ± 12.6	34.1 ± 5.1
4	27.3 ± 1.6	40.2 ± 11.0
6	16.9 ± 3.8	21.5 ± 13.0
8	13.3 ± 11.8	14.8 ± 4.5
9	9.7 ± 6.0	9.7 ± 6.5
10	14.4 ± 7.4	7.7 ± 4.3
11	12.4 ± 1.2	7.3 ± 6.8
12	11.8 ± 2.1	16.3 ± 12.4

*Each value means average ± standard error (n = 3)

Anabaena sp. PCC 7120 and *Nostoc* sp. HK-01 (Ehling-Schulz *et al.*, 1997; Tamaru *et al.*, 2005; Knowles and Castenholz, 2008; Yoshimura *et al.*, 2012). Our results proved that EPS can not protect the cells from dry heat at a temperature as high as 100°C.

There are several functions related to dry heat tolerances in a water bear; *Milnesium tradigradum*, *Richtersius coronifer* and *Ramazzottius varieornatus*, and a sleeping chironomid; *Polypedilum vanderplanki*, which are anhydrobiotic organisms (Jönsson *et al.*, 2008; Yamaguchi *et al.*, 2012; Gusev *et al.*, 2014). *Milnesium tradigradum* and *Richtersius coronifer* were exposed to space vacuum and two different UV-radiation spectral ranges for 10-days in low Earth orbit during the FOTON-M3 mission conducted by ESA, European Space Agency (Jönsson *et al.*, 2008). After the exposure, it was found that *Milnesium tradigradum* and *Richtersius coronifer* could survive, although the detailed mechanisms of their survival have not yet been elucidated. Recently, Cytoplasmic Abundant Heat Soluble protein (CAHS), and Secretory Abundant Heat Soluble protein (SAHS), were identified in *Ramazzottius varieornatus* as the function related to tolerance to extreme heat desiccation (Yamaguchi *et al.*, 2012).

Fig. 6. The amount of trehalose (A) and sucrose (B) in sample 1 and 2 (n = 3). The ratio of akinete content in sample 1 was lower than that in sample 2 (Table 3).**Table 3.** Akinete content ratio of *Nostoc* sp. HK-01 in sample 1 and 2. The ratio of akinete content in sample 1 was lower than that in sample 2.

	Sample 1	Sample 2
Exp. 1	5.22	59.89
Exp. 2	4.91	53.27
Exp. 3	3.51	62.44
Ave.	4.55	58.53
SD	0.91	4.73
SE	0.52	2.73

We have analyzed the amounts of trehalose and sucrose in two cell groups; one had a low ratio of akinete cells which was around five percent, and the other had a high ratio of akinete cells which was around 60 % (Table 3). As shown in Fig. 6, there is an exact accumulation of sucrose, particularly in the group with a high ratio of akinete cells, although there was low accumulation of trehalose. It has not been yet elucidated that sucrose may act as a compatible solute, because sucrose is an important substance for energy in all organisms. These results indicate that sucrose may have some role in dry heat tolerance, although trehalose does not have any role in heat tolerance in the case of *Nostoc* sp. HK-01. The details of the mechanisms of sucrose will have to be examined in the next step of our research.

In the present study, we have shown that *Nostoc* sp. HK-01 is far different from *Nostoc commune*, as described above. First of all, the life cycle of cells was different. For example, *Nostoc* sp. HK-01 has four types of cells and especially akinetes have high tolerance to dry heat at 100°C. Tamaru *et al.* (2005) reported that the survival rate of cells without EPS in *Nostoc commune* was remarkably reduced even under the air-dried condition. Thus, the life cycle of cells and the tolerances to harsh environments were different. Katoh *et al.* (2003) reported that the DNA sequences of the 16S rRNA gene region and *trnL* (UAA)-intron region were determined and *Nostoc* sp. HK-01 is definitely classified in *Nostoc* species. The scientific information about this strain has been accumulated, especially about high tolerances to several extraterrestrial environments and their related chemicals. This strain has been used in the 'Tanpopo mission' (Yamagishi *et al.*, 2007, 2009; Kawaguchi *et al.*, 2016). Kimura *et al.* (2015ab) reported that this strain is suitable to introduce into Mars' environment as the first organism for the preparation of manned space activities. So, we are thinking that the scientific name of *Nostoc* sp. HK-01 should be changed according to additional results in the near future.

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