Strong environmental tolerance of moss *Venturiella* under very high pressure

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**Abstract.** It was shown by the present authors group [1] that tardigrades can survive under high pressure of 7.5 GPa. In the case of land plants, however, no result of such experiment has been reported. We have extended our experiments to moss searching for lives under very high pressure. Spore placentas of moss *Venturiella* were sealed in a small Teflon capsule together with a liquid pressure medium. The capsule was put in the center of a pyrophillite cube, and the maximum pressure of 7.5 GPa was applied using a two-stage cubic anvil press. The pressure was kept constant at the maximum pressure for 12, 24, 72 and 144 hours. After the pressure was released, the spores were seeded on a agar medium, and incubated for one week and more at 25°C with white light of 2000 lux. It was proved that 70-90% of the spores were alive and germinated after exposed to the maximum pressure of 7.5 GPa for up to 72 hours. However, after exposed to 7.5 GPa for 6 days, only 4 individuals in a hundred were germinated. The pressure tolerance of moss *Venturiella* is found to be stronger than a small animal, tardigrade.

**1. Introduction**

It was shown by the present authors group [1] that tardigrades can survive under very high hydrostatic pressure, as high as 7.5 GPa, for up to 13 hours. In the case of plants, however, there are very few of such experiments have been reported. We have extended our experiments to moss searching for lives under extreme environmental condition of very high hydrostatic pressure. 

Mosses (Bryophyta) have been living on the Earth since a few billion years ago, and considered to be the earliest land plants. Most mosses are found in areas of humid atmosphere and cold to moderate
warm climate. Spores of mosses are strong against extreme environmental conditions such as long-term dried weather, in vacuum, under high pressure and at high temperature. *Venturiella sinensis* belongs to *Erpodiaceae*, being one of the popular mosses seen in moderate climate areas such as in Japan, Korea and China. The spores of *Venturiella* are spherical in shape with a diameter of about 20 µm. A plasentas, being in the size of 0.5 mm in diameter and 1 mm in length contains several ten thousands of spores. Once spores are put in a dried atmosphere, they become strong against extreme environmental conditions and remain alive for long years. When they are seeded on a humid media, they begin to germinate within one week. This moss, therefore, would be a good candidate for the search of life under very high hydrostatic pressure up to 7.5 GPa.

2. Experimental method
The present experiments searching for lives under very high pressure were performed by using a cubic anvil type high-pressure apparatus [2, 3] with which it was possible to generate high hydrostatic pressures as high as 7.5 GPa in a relatively large volume.

Specimens of spore placentas of the moss, *Venturiella* collected from the field were dehydrated by keeping them in a low humidity container for more than one week. Three to four placentas were sealed in a small Teflon capsule with an inner diameter and the length of 1.6 and 1.8 mm, respectively. As a liquid pressure medium, Fluorinate was adopted and put into the Teflon capsule together with the specimens. Figure 1 (a) shows the Teflon capsule with three placentas before the pressure was applied. It was proven that soaking in Fluorinate makes no practical influence to the life of tardigrades [1] at the cryptobiotic state. The capsule was put in the center of a cube which was made of pyrophillite with an edge length of 6.0 mm. This cube was compressed by six WC-Co anvils with a front edge length of 4.0 mm. These anvils were compressed by a 250-ton press. The pressure was determined by using a calibrated curve [2, 3] of the relation between the press load and the actual pressure established before the experiment using the pressure dependence of the superconductivity transition point of Pb. The error of the intensity of pressure was smaller than 0.3 GPa. In this apparatus the press load was controlled to keep the intensity of the pressure constant during the operation at the maximum pressure.

The pressure was increased from ambient to the maximum pressure at a rate of 0.3 GPa/min. The pressure was kept constant at the maximum pressure for 24 to 144 hours, and then, decreased down to the ambient pressure with the same rate as on increasing.

After the pressure was released, the Teflon capsule was brought out of the high pressure cell. Figure 1 (b) shows the capsule with the spore placentas after the pressure was released. The spores were
seeded on an ager medium, and cultured in an incubator for a week and then for more longer period at the constant temperature of 25 °C with white light of 2000 lux.

3. Results and discussion

As seen in Fig. 2 (a) and (b), the spores began to germinate in both control and high pressure exposed groups for up to 72 hours. The germination rates of those high-pressure exposed groups were 70-90%, which is as high as the control.

![Figure 2](image)

Figure 2. (a) Germinated spores of Venturiella exposed to the high hydrostatic pressure of 7.5 GPa for 3 days. The photo was taken two weeks after seeded, and (b) control. The inserts are in an expanded scale.

After being incubated for five months, those spores grew up to have green leaves as seen in Fig. 3. On the other hand, those spores exposed to 7.5GPa for 6 days, only 4 individuals in a hundred were germinated. The pressure tolerance of moss is found to be about three times greater than tardigrades [1].

![Figure 3](image)

Figure 3. (a) Green leaves of Venturiella experienced the high hydrostatic pressure of 7.5 GPa for 3 days. The photo was taken five months after the spores were seeded on an ager medium, and (b) in an expanded scale.
From the present experiments the effect of high hydrostatic pressure of 7.5 GPa on moss spores and their survival limit were investigated. The survival limit of the exposure time to the high pressure, 7.5 GPa is determined to be around 144 hours. We convinced that moss spores are still alive after being exposed to such a very high hydrostatic pressure of 7.5 GPa, which corresponds to the pressure at the depth of about 180 km below the surface of the Earth. This depth is equal to 1/35 of the radius of the Earth, and is in the middle of the upper mantle.

The results of the present investigation may give the possibility that mosses, Ptychomitrium [4] and Venturiella, as well as small animal, tardigrades [1] and plankton, artemia [5, 6] can travel through the space in a large meteorite, and reach the earth alive from other planet or galaxy.

4. Conclusion
It was proved from the present investigation that 70-90% of the spores were alive and germinated after exposure to the extremely high pressure of 7.5 GPa for up to 72 hours. However, after exposed to 7.5 GPa for 6 days, only 4 individuals in a hundred were germinated. The pressure tolerance of moss Venturiella is found to be much greater than tardigrade.

References
[1] Ono F, Saigusa M, Uozumi T, Matsushima Y, Ikeda H, Saini N L and Yamashita M J. Phys. Chem. Sol. 69, 2297
[2] Matsushita M, Endo S, Miura K and Ono F 2003 J. Magn. Magn. Mater., 265, 352
[3] Matsushita M, Nakamoto Y, Suzuki E, Miyoshi Y, Inoue H, Endo S, Kikegawa T and Ono F 2004 J. Magn. Magn. Matter. 284, 403
[4] Nishihira N, Shindo A, Saigusa M, Ono F, Matsushima Y, Mori Y, Takarabe K, Saini N L and Yamashita M 2009 Proc. of SMEC2009 (Miami Caribbean) presented
[5] Ono F, Minami K, Saigusa M, Matsushima Y, Mori Y, Takarabe K, Saini N L and Yamashita M 2009 Proc. of SMEC2009 (Miami Caribbean) presented
[6] Minami K, Ono F, Mori Y, Takarabe K, Saigusa M, Matsushima Y, Saini N L and Yamashita M, This conference, 29P35