Tolerance of budding yeast *Saccharomyces cerevisiae* to ultra high pressure

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**Abstract.** Our studies on the tolerance of plants and animals against very high pressure of several GPa have been extended to a smaller sized fungus, the budding yeast *Saccharomyces cerevisiae*. Several pieces of budding yeast (dry yeast) were sealed in a small teflon capsule with a liquid pressure medium fluorinate, and exposed to 7.5 GPa by using a cubic anvil press. The pressure was kept constant for various duration of time from 2 to 24 h. After the pressure was released, the specimens were brought out from the teflon capsule, and they were cultivated on a potato dextrose agar. It was found that the budding yeast exposed to 7.5 GPa for up to 6 h showed multiplication. However, those exposed to 7.5 GPa for longer than 12 h were found dead. The high pressure tolerance of budding yeast is a little weaker than that of tardigrades.

1. Introduction

It is known that proteins began to unfold around 0.3 GPa, where most bacteria and many other organisms die [1-4]. Therefore, the effects of hydrostatic pressure on biological samples have mostly been investigated within this pressure order. However, under the pressure of 0.6 GPa, which was two times higher than the pressure mentioned above, tardigrades (*Milnesium tardigradum*) were shown to survive [5]. Further extensions of the pressure to a few GPa order were made by Sharma et al. [6] and Margosch et al. [7] on some microbial activities and Horikawa et al. [8] on some small animals at dehydrated state including tardigrades. Furthermore, in our experiments on the tolerance of small animals and plants, it was shown that all the animals and plants tested up to date could withstand the very high pressure of 7.5 GPa [9-16]. We have extended these studies to a smaller size fungus, budding yeast *Saccharomyces cerevisiae*.

*Saccharomyces cerevisiae* is the common yeast used in baking bread, brewing and wine making through alcoholic fermentation. The size of the cells is 5-10 micrometers in diameter and the cells reproduce through a division process known as budding. The replicable life span of *Saccharomyces*...
cerevisiae is as short as about 2 days, being a good candidate for study of effect of hydrostatic pressure on viability of budding yeasts.

Hydrostatic pressure effects on microbial activities of Saccharomyces cerevisiae in metabolic states have mostly been investigated in the range up to 0.3 GPa [17-19]. It was shown that protein synthesis is completely blocked at 0.067 GPa [17, 20, 21], and protein unfolding, denaturation and dissociation take place reversibly at the pressure range of 0.1-0.3 GPa. It was also reported that wild-type yeast cells were killed at 0.22 GPa [22].

Tardigrades at the tun-state (dehydrated state) are more than two orders of magnitude stronger [9] than at the metabolically active state. As to the dehydrated state of the budding yeast, on the other hand, very few studies have been made to date. Summarising our data so far obtained to date [15], it was shown that the smaller the size of the living specimen is, the stronger the tolerance against high pressure. Yeast at the dehydrated state, therefore, seems to show strong resistivity against ultra high hydrostatic pressure of several GPa order.

2. Experimental Procedure

As for the specimens, commercially sold “dry-yeast” for baking (Oriental Yeast Co., Ltd., Nisshin Seifun Group) was used. Several pieces of dry-yeast, budding yeast, Saccharomyces cerevisiae were put in a small teflon capsule with an inner diameter and length of 1.6 and 1.8 mm, respectively. As for liquid pressure medium, Fluorinate PC72 (perfluorocarbon, Sumitomo 3M) was chosen and put into the capsule together with the specimens. It was proven [6, 9] that soaking in 100% fluorinate has no practical effect on the life of living specimens. The capsule was placed in the center of a cube which was made of pyrophyllite with an edge length of 6.0 mm. Figure 1 (a) shows the yeast samples in a teflon capsule which was placed in the centre of a pyrophyllite cube.

To generate hydrostatic pressure up to 7.5 GPa, a cubic anvil press was used. The pyrophyllite cube was placed at the centre of six tungsten-carbide 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 of the relation between the press load and the actual pressure established before the experiment. The error of the intensity of pressure was smaller than 0.3 GPa. In the present apparatus, the press load was controlled automatically to keep the intensity of the pressure constant and to maintain maximum pressure during the operation [9-16].

**Figure 1.** (a) Yeast sample in a teflon capsule (before exposure), (b) four of the six anvils with the high pressure cell and (c) yeast sample in the teflon capsule after exposure to 7.5GPa for 24 h.
The pressure was increased from ambient to the maximum pressure of 7.5 GPa at a rate of 0.3 GPa/min. From our former experiments observing temperature dependence of magnetic susceptibility on increasing and decreasing pressure using the same high pressure equipment [23], it was proven that the temperature of the sample had been kept constant within a relative error of about 1 K. The pressure was kept constant at maximum pressure for various duration of time from 2 to 24 h and then brought down to the ambient pressure reversing the same rate of increments used for increasing. Those high pressure experiments were made at room temperature. After the pressure was released, the specimens were brought out from the teflon capsule. Four of the six-anvils are shown in figure 1 (b) with the pyrophylite cube, and in (c) the teflon capsule with the dry-yeast pieces after the maximum pressure of 7.5 GPa for 24 h.

After exposure to high pressure, the specimen of several pieces of the budding yeast, “dry-yeast” were taken out of the teflon capsule and cultivated on a potato dextrose agar (PDA, Merck-Askul 6-8813-02) for a few days at a constant temperature of 303 K. Then, their growth was investigated under a microscope.

3. Results and Discussion
It was found that the yeast exposed to 7.5 GPa for up to 6 hours were grown as much as the control, while no growth was seen in those exposed to high pressure for more than 12 hours. Observed microscope images for the control, which was not exposed to high pressure, and exposed to 7.5 GPa for 2 and 6 hours are shown in figure 2.

Figure 2. Yeast, *Saccharomyces cerevisiae* observed before and after high pressure exposure; (a) control, (b) after exposure to 7.5 GPa for 2 hours and (c) for 6 hours.

There are no significant differences in the images between the control and the high pressure-exposed yeast for up to 6 hours. On the other hand, no such image for yeast fungus was seen in those exposed to high pressure for longer than 12 hours.

To investigate the alcoholic fermentation capability of the high pressure-exposed yeast, the samples were cultivated further in test tubes with YPD (yeast extract 1%, Peptone 2%, Dextrose 2%) agars for 3 days. Accurate quantifications of ethanol concentration in the YPD were made by using a commercially available test kit (F-kit, Boehringer Mannheim) combined with a photo spectrometer.
The results are shown in figure 3. As seen in this figure, the alcoholic fermentation capabilities of high pressure-exposed yeast for up to 6 hours were as high as that of the control. On the other hand, no alcoholic fermentation was detected from the yeast exposed to high pressure for more than 12 hours.

The results of the present experiments for the survival of yeast exposed to 7.5 GPa are summarized in figure 4 as a function of exposed time. In this figure the survival rate for tardigrades [9] were also shown for comparison. The high pressure tolerance of yeast fungus is a little weaker than that of tardigrades. This result is quite inconsistent with the tendency of our data so far obtained to date [15] that the smaller the size of an individual living sample is, the stronger the tolerance would be to high pressure.

Figure 3. Alcoholic fermentation capability of yeast exposed to 7.5 GPa for 2, 6 and 12 h. The values are normalized with that of the control.

Figure 4. The normalized alcoholic output of yeast as a logarithmic function of exposed time to 7.5 GPa. The survival rate for tardigrades (dotted line) [9] is also shown for comparison.

For spores of mosses, Ptichomitrium and Venturiella, with their average diameter of 25-30 microns, it was shown [12, 13] that they could tolerate 7.5 GPa for as long as 144 h. Considering the smaller size of yeast, with their average diameter of 5-10 microns, we initially expected them to withstand the high pressure of 7.5 GPa longer than these mosses. However, the present results showed that the survival limit of yeast under high pressure was shorter than 12 h, which was more than one order of magnitude shorter than expected. The reason why the high pressure tolerance of yeast is much weaker than expected is not clear yet.

Considering the fact that for monomeric proteins unfolding takes place at 0.4-0.8 GPa, and this pressure is the likely maximal pressure for survival of organisms at metabolic state [24], it remains a big question why yeast in dried state can tolerate such a very high hydrostatic pressure of 7.5 GPa.
4. Conclusion

The tolerance of budding yeast *Saccharomyces cerevisiae* to the very high pressure of 7.5 GPa was investigated by using a cubic anvil press. There are no significant differences in the images between the control and the high pressure-exposed yeast for up to 6 hours. The alcoholic fermentation capability for high pressure-exposed yeast for up to 6 hours was as high as that of the control. On the other hand, no such image for yeast was seen nor any alcoholic fermentation was detected from the yeast exposed to high pressure for longer than 12 hours. The high pressure tolerance of yeast is a little weaker than that of tardigrades.

These results are inconsistent with the tendency of our data so far obtained to date that samples of smaller size have stronger tolerance to high pressure.

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