Functional Analysis of Coenzyme A Disulfide Reductase in the Thermo-Acidophilic Red Alga, *Galdieria sulphuraria*

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【Introduction】

Cyanidiophyceae inhabit extreme environmental conditions such as hot, toxic, and acidic environments where other organisms cannot grow efficiently. They even thrive in pure CO_2 conditions [1]. Among Cyanidiophyceae, *Galdieria sulphuraria* shows the highest tolerance to acid, salt, and CO_2 stresses. However, the mechanism to adapt to a wide range of stresses is still not fully understood.

From a previous study done by our laboratory, Gasu_05470, a homolog of Coenzyme A (CoA) disulfide reductase (CoADR), was identified as a highly expressed protein under high CO_2 condition by LC-MS/MS analysis. CoADR is known as a regulator of cellular redox by using CoA. Most organisms use glutathione reductase (GR) to keep the cellular redox homeostasis under stress conditions, while some anaerobic bacteria use CoADR instead of GR [2]. The possession of CoADR in addition to GR suggests that *G. sulphuraria* uses two cellular redox systems (glutathione-GR and CoA-CoADR) to deal with environmental stresses including high CO_2.

In this research, I examined the changes in the expression of *Gasu_05470* and *GR* genes in response to several stresses (high CO_2, low O_2, and low temperature) to elucidate the function of Gasu_05470 in *G. sulphuraira*. Furthermore, since *Cyanidioschyson merolae* in Cyanidiophyceae does not have CoADR homolog, I will characterize the CoADR overexpression strain in *C. merolae*, to clarify its function *in vivo*.

【Materials and Methods】

Algal culture and sampling

G. sulphuraria 074W (NIES-3638) cells were grown in modified Allen's Medium (pH 2.5) at 40 ℃ under ordinary air condition [3]. For the sampling, the cells were grown until the mid-exponential growth phase (OD_{750} = 0.5-0.8), then transferred from the optimum growth condition (air; 40 ℃) to the certain stress conditions (100% CO_2, 100% N_2, and 28 ℃).

Total RNA extraction and purification

Total RNAs were extracted from the cells that collected at each time point (0, 0.5, 1, 3, and 6 hour) after the transfer to each stress condition and purified using PureLink® RNA Mini Kit (Invitrogen™) including DNAse treatment.

Reverse Transcription and PCR amplification

Reverse transcription was performed using ReverTraAce® qPCR RT Master Mix (TOYOBO). After the PCR amplification, PCR products were analyzed by electrophoresis.

【Results and Discussion】

The expression of *Gasu_05470* gene was strongly upregulated within 1 hr after the transfer to 100% CO_2, whereas the expression of *GR* gene was not changed (Figure 1). A similar result was obtained by the transfer from air to low O_2 (100% N_2) condition. On the other hand, by the shift from 40 ℃ to 28 ℃, both expressions of *Gasu_05470* and *GR* genes were upregulated.

From these results, we hypothesize that CoADR-CoA system maintains cellular redox in cooperation with GR-glutathione system under aerobic condition, while under anaerobic condition, CoADR-CoA system is a main redox regulatory system in *G. sulphuraria*.

To test the functional complementation of GR by Gasu_05470 *in vivo*, we will examine the Gasu_05470 overexpressed transformants in the wild type and GR knock-out strains of *C. merolae*. This result will be reported in the presentation.

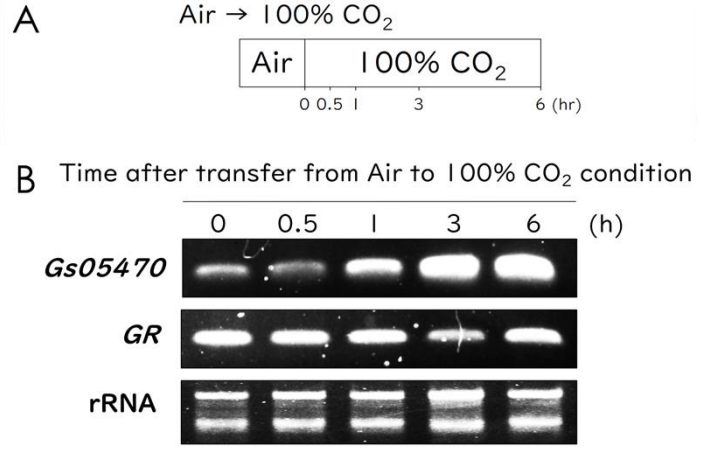

Figure 1. Expression analysis of *Gasu_05470* and *GR* genes in *G. sulphuraria* cells transferred from air to 100% CO_2. Experimental Scheme (A). RT-PCR analysis of *Gasu_05470* and *GR* genes (B)

【Conclusion】

The followings can be drawn from the present research:

- *Gasu_05470* gene was highly expressed under high CO_2 condition.
- Expression of *Gasu_05470* gene was induced by CO_2, N_2, while the expression of GR gene was not changed.
- Expressions of both *Gasu_05470* and *GR* genes were upregulated in response to low temperature.
- Gasu_05470 seems to work as a main redox regulator under anaerobic condition.

【References】

[1] Seckbach *et al.* (1970). Nature 227, 744-745.
[2] Boylan *et al.* (2006). Molecular Microbiol. 59, 475-486.
[3] Minoda *et al.* (2015). Appl Microbiol Biotechnol 99, 3, 1513-1519.