Effects of high hydrostatic pressure on metabolisms of bathypelagic bacteria

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To assess the effects of high hydrostatic pressure on metabolisms of bathypelagic bacteria, we developed a portable and relatively inexpensive high-pressurizing incubation system. The system mainly consists of a conventional HPLC pump, shut-off valves, gauges and HPLC column-like stainless steel pipes as the pressurizing vessels in which FEP-based incubation vessels were pressurized. The maximum hydrostatic pressure that could be stably maintained was 30 MPa. No solution leakage between the pressure-transmitting medium and the incubation samples was observed during pressurization. The system was useful in providing simultaneously multiple pressure and conditions of incubation in one experiment. The performance and portability of the system is suited particularly for field survey on board to investigate bacterial metabolisms and related biogeochemical processes in bathypelagic environments. We executed on board measurements of production rates of natural bacterial assemblages at *in situ* hydrostatic pressure and temperature in ship’ s radiation-controlled laboratory. Seawater samples were collected from bathypelagic depths (500-3000 m) in the Pacific Ocean between 0.5˚N and 50˚N along 170˚W during the KH14-3 cruise of *R/V Hakuho-maru*. Bacterial production rates measured by a ^{3}H-leucine incorporation method at *in situ* high pressure were 1.1 to 12.4 times higher than those at atmospheric pressure. This result strongly suggests that bacterial production rate is substantially underestimated at normal atmospheric pressure in comparison with *in situ* conditions.

As an example of application of the pressurizing incubation system, we investigated the impacts of high CO_2 exposure with a potential leak from the seabed of carbon dioxide capture and storage (CCS) sites on a strain of *Pseudoalteromonas* sp. isolated from 2000 m depth of the western North Pacific Ocean. Combined effects of high hydrostatic pressure and high CO_2 concentration were tested from atmospheric pressure to 30 MPa and from 0.1% to 20% of CO_2. In acidification experiments, gas exchange through the semi-permeable FEP membrane could cause changes in pH of the incubated samples. However, this was fixed by using the pressure-transmitting media equilibrated to appropriate CO_2 concentrations in advance. The specific growth rate of *Pseudoalteromonas* sp. isolate at 0.1% CO_2 was substantially high at 10 MPa in comparison with atmospheric pressure. Although the growth was completely inhibited with 20% CO_2 at atmospheric pressure, the bacteria exhibited high tolerance for high CO_2 even at 20% between 10 MPa and 30 MPa. This result suggests the possibility that the response of bathypelagic bacteria to high CO_2 is overestimated at normal atmospheric pressure in comparison with *in situ* conditions. Various technologies have been developed for utilizing resources and energy of the bathypelagic ocean environments, including CCS and exploitation of deep-sea mineral ore resources. A variety of perturbations not just with high CO_2 is possible in such industrial usage of marine environments, including exposure to heavy metals and chemical substances, as well as physical disturbance. Usage of a high-pressure incubation device as presented here is highly recommended to realistically examine the impacts of various environmental perturbations on bathypelagic bacterial communities.

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