Analysis of gene expression of aquaporins inducing stress tolerant in the Antarctic midge, *Belgica antarctica*

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Antarctic terrestrial environment is very harsh, and the numbers of terrestrial species is very limited. The Antarctic midge, *Belgica antarctica* is a terrestrial insect living in the Antarctic Peninsula and South Shetland Islands. They have a two-year life cycle, and they spend most of the period as larvae. Their habitat is in the coastal mosses and mud, gets wet by sea spray in summer, and covered with snow and ice in winter. It is known that larvae of this species are tolerant to various environmental stresses such as drying, high salinity, low temperature and freezing. In addition, it has shown that dehydration induce enhancement of cold tolerance in this species. The intracellular movement of water is caused by aquaporin (AQP) that is a protein distributed on the cell surface. Some experiments using AQP's inhibitor, $HgCl_2$, show that AQP is involved in cold tolerance. By genome sequence, this insect has 6 AQP genes, *Drip1, Prip1, Lhip1, Rpip1, Rpip2, bib*. In this study, I focused on aquaporin as a factor inducing cold tolerance of *Belgica antarctica*. In order to investigate the role of aquaporin in cold tolerance, the mRNA levels in whole body of 4^{th} instar larvae were examined.

First, I set three conditions of drying, cryoprotective dehydration, and high salinity. For drying conditions, larvae were placed in the slow dehydration conditions (RH 74%, 4°C) for 48 h (SD48h) or 5 days (SD5d). For cryoprotective dehydration (CD), larvae were gradually cooled from -0.6 °C to -3°C for 5 days and left at -3°C for 10 days. For high salinity, larvae were soaked in the 4× Coast's solution, a concentrated saline solution, for 2 days. Survival rate and water loss under these conditions were also examined. The survival rate was 90% under SD5d, and 100% under other conditions. The average of water loss was, in decreasing order, 48% in SD5d, 45% in CD, 21% in SD48h, 19% in the 4× Coast solution, and -1.7% in the 1× Coast solution.

Next, AQP mRNA levels were examined in these experimental animals by Real time PCR (RT-PCR). In the present study, I focused on *Drip1*, *Prip1*, *Rpip1* and *bib*. The mRNA levels of *Drip1* and *Prip1* were significantly higher in the CD group than in the control. The *Drip 1* and *bib* mRNA levels were significantly decreased in the 4× Coast group than in the control. Despite the high water loss at SD48h and SD5d, mRNA levels of these genes were not significantly different from control. There was no difference in mRNA levels of *Rpip1* among these conditions. *Drip 1*, *Prip1*, and *bib* are thought to have some function under cryoprotective dehydration and high salinity.