学位論文の要約

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学位論文の題名

Studies on vitamin B₁₂ supplementation to reduce turbidity stress on Cladoceran zooplankton

(ミジンコ類の濁度変化ストレス軽減に対するビタミンB12の効果)

学位論文の要約

Recently, there is increasing concern on the occurrence of extreme weather and high intensity of precipitation related to ongoing climate change. The condition will exacerbate with sediment loading from the land that contributes to the water clarity in aquatic ecosystem. Filter-feeder organisms, such as Cladoceran zooplankton, are one of the animals that susceptible for any particles loading since they do not have capability to select unwanted food. To deal with fluctuating environment and sudden stress condition, the fitness of organism depends on their capability to maintain oxidative metabolism. There is growing evidence that in the laboratory scale as well as natural environment, bacteria has nutritional role for zooplankton in many ways. One of nutrients exclusively produced by bacteria and readily available in the water is vitamin B_{12} that also known for antioxidant, decreasing anxiety, and a key nutrient for normal development and growth. This study hypothesized that the presence of vitamin B_{12} both in the ambient water or within the food can maintain zooplankton performance to face fluctuating environment such as turbidity. The purpose of this study is to investigate the linkage of bacteria and two freshwater zooplankton, *Moina macrocopa* and *Daphnia magna*, in facing environmental change, such as turbidity, through vitamin B_{12} supplementation in the food and/or grown media.

The experiment was conducted in individual basis using 6-well microplates, under controlled condition (12L:12D, $20\pm1^{\circ}$ C). During the experiment, the test animals were fed by *Chlorella vulgaris* that cultured using modified f/2 medium (without vitamin B₁₂). Kaolin clay (Al₂Si₂O₅(OH)₄) were used as turbidity material, by diluting with distilled water until several turbidity levels applied (0, 15, 50, 100, 600, and 1200 NTU). To prepare vitamin B₁₂ solution, Crystalline cyanocobalamin was used and diluted with distilled water until several doses applied (0, 5, 25 µg/l for *M. macrocopa* and 0, 5, 15, 30 µg/l for *D. magna*). For *M. macrocopa* experiment, commercial *Chlorella* that already contained vitamin B₁₂ in the cells were also used as references. The animals were exposed by turbidity for 3 hours start from <24 h age old (1st instar juvenile) to their natural death. The observation was conducted daily covered information about the lifespan, number of offspring, time when the first reproduction started, offspring size, and offspring sex.

The absence of vitamin B₁₂ in the food and/or grown media together with short-time turbidity changed

had decreased lifespan, offspring number, offspring size, and delayed the first time of reproduction of *Moina* macrocopa (P<0.05). The greatest performance was recorded in the treatment groups that fed on commercial *Chlorella*. The results indicated that the presence of vitamin B₁₂ in the water and/or food help *Moina* to deal with environmental change. In addition, vitamin B₁₂ was better uptake through the food than in the ambient water for *M. macrocopa*. Although short-time turbidity changed had negatively affected the reproduction of *Moina*, all offspring found in the experiment are recorded as female, indicated that *Moina* can maintain parthenogenetically reproduction when exposed in the maximum 100 NTU or 600 NTU with higher doses (25 $\mu g/l$) of vitamin B₁₂ supplementation.

Different response was found in *Daphnia magna*. Short-time turbidity changed were not significantly affected the lifespan and reproduction of the animal. However, the mothers live in the highest turbidity showed more frequent reproduction compare to the mothers live in the lower turbidity and clear water (P<0.05). In addition, supplementation of vitamin B₁₂ in the ambient water showed increasing trend (although it is not significant) of offspring number until the 5th of reproduction. In *D. magna*, the effect of short-time turbidity changed together with the absence of vitamin B₁₂ supplementation was found in the offspring condition although it was found very subtle. The cumulative ratio of male and malformation offspring was vitamin B₁₂ and malformation offspring were lessening recorded following the increasing dose of vitamin B₁₂ in the water. *D. magna* can maintain parthenogenetically reproduction under sudden turbidity changed with the help of vitamin B₁₂ (15 and 30 µg/l) presence in the ambient water.

To conclude, it became clear that the reproduction of Cladoceran zooplankton had affected by turbidity changed even in a short-time period and the presence of vitamin B_{12} in the ambient water can help the animals to deal with such environmental change. In addition, this finding suggested that in natural environment, bacteria as the producer of vitamin B_{12} , might have nutritional role for higher trophic level organisms in changing environment. The present study will extend our knowledge that resilience of zooplankton to deal with environmental change can be obtained by the help of key nutrients produced by bacteria.