南極産菌類及び藻類由来の不凍タンパク質の機能解析

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Antifreeze activities of fungi and sea ice diatoms from Antarcica

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Antifreeze protein (AFP) is a key substance that contributes for animals, plants and microorganisms adapting cold environment by its unique ability of binding on the ice crystal and ice growth inhibition. This ability of AFP leads to a depression of freezing point of AFP solution but without changing the melting point. This depression of freezing point is termed thermal hysteresis (TH) and used as an indicator of antifreeze activity. AFP also modifies ice crystal to specific shapes within TH gap.

Many fungi are living in Antarctica and survive the harsh climate. To understand the biologic mechanisms of fungal cold-adaptation, we examined extracellular antifreeze activity of over 30 species of fungi that were isolated from Antarctic soil, excrements of penguins, samples of lakes and marshes. We found the antifreeze activity and determined extracellular AFP of a basidiomycetous yeast *Leucosporidium antarcticum*. Molecular weight and western-blotting analysis of this AFP suggested it is similar with a known hyperactive AFP from basidiomycetous snow mold *Typhula ishikariensis*. We also found one species of an ascomycete, *Antarctomyces psychrotrophicus* produced extracellular AFP. This fungus can be isolated from wide ranges of Antarctica including King George Island, South Shetland Islands, Prydz Bay (Zhongshan station) and Lützow-Holm Bay (Syowa station). Extracellular AFP from *A. psychrotrophicus* (AnpAFP) is the first AFP discovered from ascomycota. AnpAFP showed 28kDa on SDS-PAGE and several peaks between 21,000 and 22,000 by MALDI-TOF/MS. N-terminal sequence of AnpAFP was determined and did not show any similarities with known fungal and other AFPs. Glycoprotein analysis and western-blotting also suggested that AnpAFP was a different molecular type to basidiomycetous AFPs. Functional analysis of AnpAFP was performed by observing a single ice crystal utilizing our in-house-built photomicroscope system. We determined the TH of AnpAFP and AFP from basidiomycete (figure). AnpAFP showed almost the same antifreeze activity with typical AFPs from fish. AFP from basidiomycete showed over 2 times higher activity than AnpAFP. Ice shape modification of these AFPs from basidiomycetes and ascomycetes was also quite different, which suggests these two kinds of AFPs might bind to different surfaces of ice crystal. These characteristic analysis data of AnpAFP reveal that AFPs from basidiomycetes and ascomycetes are biologically and functionally diversified. Besides these, one hyphochytridiomycete had TH activity both in intra and extracellular space. It is still unclear about this AFP but one has been proved is that it did not react with anti-basidiomycetous AFP antibody.

We also determined the antifreeze activity about Antarctic sea ice diatom *Navicular glaciei* which was isolated from brown-colored sea ice near McMurdo Station. AFP from *N. glaciei* (NagAFP) shares nearly 50% of similarities of amino-acid sequence with AFP from basidiomycete (*T. ishikariensis*), ice-binding proteins from Antarctic bacteria and other sea diatoms. We found that NagAFP showed very high TH activity (2.5°C) even at very low concentration (0.5mM) (figure). The ice modification of NagAFP suggests NagAFP could bind to multiple surfaces of ice crystal. Further more, we found that the ice-binding ability of NagAFP showed dependence on binding rate and binding time onto the ice

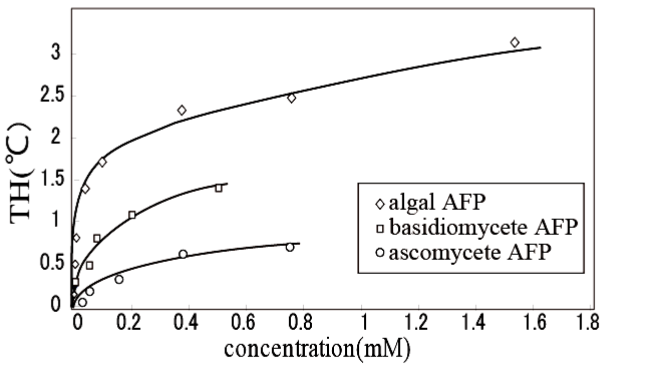

Figure TH activity of AFPs from sea ice diatom(◇), basidiomycete (□), and ascomycete (○) dependent on protein concentrations.

crystal. The longer-time-binding leads to higher TH value. The high ability of NagAFP of binding on ice and inhibiting ice growth means that NagAFP is expressed to resist the freezing at sub-zero temperature consequently protecting the cells.

In this study, we isolated over 300 isolates of fungi and relative taxa form Antarctica, and they almost belong to basidiomycetes and ascomycetes. Only a few of them produced extracellular AFPs. For cold-adapted fungi, several basidiomycetous snow molds were reported to produce extracellular AFPs. Our results revealed that not only basidiomycetes, Antarctic ascomycete produced different molecular type of AFP and Antarctic hyphochytridiomycetes are also capable of producing AFP for freeze resistance or freeze tolerance process. The evolution of AFPs within fungi kingdom developed to different molecular types dependent on their phyla. AFPs from fish, insects and plants also diversified to different molecular types. However, NagAFP from sea diatom had sequence similarities with fungal and bacterial AFPs. This is the first case of gene transfer crosses the kingdom border line of AFP consequently structural and functional similarities were discovered. Since NagAFP showed high antifreeze activity enough to resist the cell freezing in sea water, other AFPs with sequence similarities might have the same ability of antifreeze. This high ability of freezing resistance could be the common way for microorganisms expressing this type of AFP to adapt low temperature.

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