AFP proteins from psychrophilic yeast of the genus *Glaciozyma* and their biotechnological potential

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Abstract

The subject of the research conducted as part of this doctoral dissertation was the psychrophilic yeast of the species *Glaciozyma martinii* 186, which serves as a source of extracellular antifreeze protein (AFP). This strain was isolated from the waters of Admiralty Bay on King George Island in the South Shetland Islands archipelago. It belongs to the collection of Antarctic microorganisms maintained by the Institute of Molecular and Industrial Biotechnology at Lodz University of Technology.

In the course of the research, conditions for the biosynthesis of the native GmAFP protein produced by the *G. martinii* strain were developed, and the protein was subsequently purified using ice-affinity chromatography. Electrophoretic analysis confirmed the presence of the target protein with a molecular weight of approximately 27 kDa, which, after PAS staining, was identified as a glycoprotein. Differential scanning calorimetry confirmed the thermal hysteresis activity of GmAFP. Another characteristic activity of this type of protein, ice recrystallization inhibition, was demonstrated using light microscopy with a cooling stage. Preliminary studies on the cryoprotective role of GmAFP during the freezing process of carrots were promising. In the presence of antifreeze protein, less cell destruction was observed after thawing the vegetable, along with a reduction in the loss of carotenoid pigments from the cells. These findings provided a rationale for increasing the biosynthesis efficiency of this metabolite. To this end, attempts were made to isolate the gene encoding this protein from the native host. Unfortunately, designing primers based on the sequence of the closely related organism *Glaciozyma antarctica* did not result in amplification or detection of the desired *gmafp* gene.

To better understand the functionality of AFP proteins, further studies were conducted involving the synthetic *gaafp* gene derived from the psychrophilic yeast *Glaciozyma antarctica* PI12. *Gaafp* was successfully expressed in the methylotrophic yeast *Pichia pastoris* GS115. The highest concentration of GaAFP protein was obtained after a 3-day submerged culture, carried out in an expression medium with 1% methanol induction every 12 hours at 28°C. The recombinant protein, with a molecular weight of approximately 25 kDa, was purified using ice-affinity chromatography, and its ice recrystallization inhibition activity was confirmed using microscopy.

As part of the study on the application potential of the GaAFP protein, it was demonstrated that this protein can be successfully used as a cryoprotectant in the food industry. Adding this biocomponent to vegetables or fruits (including, for example, carrots, kohlrabi, and blueberries) prior to freezing positively impacts the structure of thawed cells and reduces water

loss from their interiors. The effect is comparable to that of the commonly used cryoprotectant, glycerol. However, the latter can alter the taste of food and is therefore not always suitable for use in the food industry. Moreover, the presence of the GaAFP protein improves the viability of *Saccharomyces cerevisiae* yeast cells during frozen storage. This strategy allows for the extension of their storage period while ensuring stable substrate reserves for the production of various types of food.

As part of the study, in vitro experiments were conducted using cell lines simulating the human digestive system (Caco-2 and HT-29 cell lines), which demonstrated that the GaAFP protein is not cytotoxic to these cells at the maximum tested concentration of 0.4 mg/ml after 24 hours of incubation. Moreover, using mouse macrophages RAW 264.7, the effect of GaAFP on nitric oxide (NO) secretion, one of the main mediators of chronic inflammatory processes, was assessed. It was found that GaAFP does not affect nitric oxide secretion in either LPS-stimulated or non-stimulated cells.