**TITLE:** Freezing tolerance of AFP-producing microorganisms isolated from different Antarctic ecosystems

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## **ABSTRACT:**

Antarctic microorganisms are widely known to present a myriad of adaptations to survive the extreme cold Antarctic environment. One of the adaptation mechanisms to freezing conditions is the production of antifreeze proteins (AFP), a group of proteins capable of interfering with the ice formation and its recrystallization, providing cellular resistance to low temperatures and freezing. The study of AFP-producing microorganisms can lead to innovation in anti-freezing technologies, with wide applications in the food, agricultural and pharmaceutical industries. The objective of this study was to characterize a collection of AFP-producing microorganisms in terms of tolerance to freezing. The use of Antarctic samples as a source for AFP-producing bacteria is highlighted by the natural characteristic of the environment, such as ice, snow, soil and permafrost, which are subjected to permanent or seasonal freezing. Bacteria and yeasts previously isolated and already identified as AFP producers were used. Initially, the microorganisms were morphologically characterized and their tolerance to freezing was tested through counting colony-forming units before and after freezing. Regarding morphological characterization, 75 isolates were Gram negative and 24 were Gram positive with a predominant form of cocci. Regarding freezing tolerance, a total of 43 isolates were tested and of these 27 had a survival rate above 50% with the highest percentage for the isolate P63 with 100%. Regarding the freeze-resistant isolates, 18 of them are gram negative, 6 are positive, and 1 yeast tested positive. Molecular identification data of rRNA16S showed a diversity of freezeresistant genera such as Arthrobacter, Pedobacter, Paenibacillus, Planococcus halocryophilus, Salinibacterium and Psychrobacter. The results obtained so far show us that the microorganisms in Antarctica are tolerant to freezing and that they can indicate the production of antifreeze proteins.

KEYWORDS: Antarctica, antifreeze proteins, freezing, microorganisms

**DEVELOPMENT AGENCY:** Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina - FAPESC