

TITLE: Bioprospection of Antifreeze Proteins (AFP) from Antarctic bacteria aiming biotechnological applications

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ABSTRACT:

About 80% of the Biosphere is at temperatures below 5 °C, which includes most of the ocean, sea ice, deep-sea sediments, mesosphere and stratosphere, as well as alpine and polar regions. Permanently or seasonally cold ecosystems harbor microorganisms that survive with low liquid water available for metabolism, as well as several freezing resistant species. Some proteins, known as antifreeze proteins (AFP), are able to inhibit the formation of intracellular ice crystals. These proteins act modifying the structure of crystals during ice formation, lowering the freezing point, and inhibiting the recrystallization activity. The objective of this study is to isolate and identify Antarctic bacterial strains that produce AFP, characterizing the inhibition of recrystallization (IR) and the thermal hysteresis (TH) effect of the proteins aiming biotechnological applications. Antarctic soils, permafrosts and mosses samples were collected on King George Island, Antarctica, during previous expeditions of the Brazilian Antarctic Program (CNPq/PROANTAR) during 2015-2017 and kept frozen at -20 °C. Isolation of bacterial strains was done using R2A media (100% and 10% [w/v]) and incubated at 6 °C and 25 °C under aerobic and anaerobic conditions. Bacterial colonies were randomly selected based on morphology and subjected to a BOX-PCR for strain differentiation. From these, 117 unique strains were selected for identification using 16S rRNA sequencing. The initial screening for AFP producing strains was done by estimating the survival rate after freezing a $\sim 1.10^7$ cell.mL⁻¹ suspension on phosphate saline buffer for 1 day at -20 °C. In general, from 24 tested strains, cell viability decreased 1 log after freezing. The isolate C300/4, obtained at 25 °C from a soil sample, showed no decrease in cell viability after the freezing treatment. The results of these screenings indicate the potential of Antarctic bacteria as sources of AFP production. The next step will be the selection of strains that produce AFP through the IR experiment, followed by the characterization of in the TH effect of the selected AFP.

KEYWORDS: Antifreeze proteins, Antarctica, bioprospection, recrystallization, thermal hysteresis, extremophiles

DEVELOPMENT AGENCY: CNPq-PROANTAR, CAPES