

# Comparative genomics in the search for Antifreeze Proteins in *Metschnikowia australis*

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## Abstract

*Metschnikowia australis* is a marine yeast confined to the Antarctic costal region. Conversely, its most related species, *Metschnikowia bicuspidata*, is worldwide distributed, but rarely retrieved from Antarctica's seas. Besides global adaptations to life at low temperatures, many psychrophilic organisms, from bacteria to vertebrates, have independently evolved protein coding genes that directly interfere with the ice formation process. These genes are collectively called Antifreeze Proteins (AFPs), consisting of a diverse class that have innumerable biotechnological applications, from frozen food industry to organ preservation for transplants. As polyphyletic, AFP genes are harder to find with traditional sequence similarity search tools in less characterized taxa, as *Metschnikowia*. One *M. australis* specimen was isolated by the MycoAntar project and, due to its ability to survive freezing, it was selected for further studies by our group. We have sequenced and assembled the *M. australis* genome, for further genomic investigation. As *M. bicuspidata* genome was already available, we devised an strategy to predict Open Reading Frames (ORFs) on both genomes and select those exclusive to *M. australis*, which could potentially code new AFPs. The 249 ORFs exclusive to *australis* were then submitted to three machine learning based AFPs classifiers, in order to select the most promising candidates for in vitro expression analysis. Primers were designed for 17 selected candidates, and these are now being used to probe both yeasts transcriptome after freezing stress. The candidate ORFs that are proven to be expressed will be further characterized. Genomic deletion and heterologous expression techniques will be used to confirm their relation to freezing survival.

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