





## Role of metal ions in cold adaptation: functional and structural characterization of an Antarctic esterase

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## Abstract:

The production of cold active enzymes is one of the main strategies developed by psychrophilic organisms to survive in cold environments. Generally, these enzymes show high activities at low temperatures coupled with high structural flexibility and thermolability. Esterases are ubiquitous enzymes that catalyse the hydrolysis of ester bonds producing a carboxylic acid and an alcohol as products. Cold active esterases are of interest in a multitude of biotechnological applications such as food processing, detergents, and bioprocessing.

Here, we report the biochemical and biophysical characterization of an esterase identified in the genome of the Antarctic bacterium *Marinomonas* sp. ef1 (*M*-Est). This enzyme is able to hydrolase esters containing acetate groups, has an optimum temperature of catalysis of 5°C and it is thermolabile. Interestingly, addition of  $Mn^{2+}$  ions induce a conformational change in *M*-Est, improving its catalytic activity and thermal stability. To investigate this peculiar behaviour, we combined an array of computational, biochemical, biophysical, and rational design techniques to identify the  $Mn^{2+}$  binding site, which is localized on the enzyme surface close to active site. Overall, our results suggest that the coordination of  $Mn^{2+}$  ions could contribute to the cold adaptation mechanism of *M*-Est.