Extreme marine and coastal environments as a source of glycoside hydrolases involved in the degradation of oligosaccharides and polysaccharides

A. Marchetti[1], M. Orlando[1], L. Bombardi[2], C. Christakis[4], V. Tsopanakis[3], P. Sarris[4], I. V. Pavlidis[3], S. Fusco[2], M. Mangiagalli[1], M. Lotti[1]
E-mail: *a.marchetti20@campus.unimib.it*

[1] Department of Biotechnology and Biosciences, University of Milano Bicocca, Milano, Italy
[2] Department of Biotechnology, University of Verona, Verona, Italy
[3]Department of Chemistry, University of Crete, GR
[4]Department of Biology, University of Crete, GR

Abstract

Extreme marine and coastal environments, such as polar regions and hypersaline habitats, force bacteria to experience and counteract a variety of stressful conditions, such as low temperatures, salt stress, and low nutrient availability. Under these harsh conditions, hydrolytic enzymes such as glycoside hydrolases (GHs) play a crucial role in the degradation of polysaccharides, which serve as carbon and energy sources for microorganisms.

Here we report our studies on different GHs from bacteria living in different extreme environments: *Marinomonas* sp. ef1, an Antarctic bacterium able to grow at low temperatures, and *Bacillus altitudinis* strain CML04, an endophytic halotolerant bacterium isolated in Crete Island.

The Antarctic bacterium *Marinomonas* sp. ef1 possess three different GHs belonging to family 3, namely M-GH3_A, M-GH3_B and M-GH3_C, which have different architectures and low sequence identity. While M-GH3_C was produced as an insoluble and inactive protein, M-GH3_A and M-GH3_B show different thermal and structural properties: M-GH3_A turns out to be a *bona fide* cold-active enzyme, while M-GH3_B shows mesophilic-like properties. Moreover, M-GH3_A is a promiscuous β-glucosidase, mainly active on cellobiose and cellotetraose, whereas M-GH3_B is a xylanase active on xylan and arabinoxylan.

The mediterranean bacterium *Bacillus altitudinis* strain CML04 displays the ability to degrade xylan-based polysaccharides and, moreover, enhances degradation activity in presence of salinity stress. Genome mining analyses identifies different GHs putatively involved in the degradation of xylan, and here we report the discovery and characterization of two extreme halotolerant xylanases belonging to GH family 11 and 30. These two enzymes are both active on xylan-based polysaccharides, have different biochemical and structural properties and, moreover, can to tolerate salinity stress up to 2,5M. Future analyses will help us to understand how the expression of these enzymes is affected by salinity stress and how these enzymes degrade xylan polysaccharides, in order to define the physiological role of these xylanases.