UNIVERSIDADE DA CORUÑA

# Identification and characterization of a novel thermostable β-galactosidase discovered through functional metagenomics

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## 1. INTRODUCTION

β-galactosidases catalyze the hydrolysis of lactose to glucose and galactose and they have drawn considerable interest from the biotechnological industry for the production of low-lactose milk and the revalorization of whey. Furthermore, some β-galactosidases can transfer the galactosyl residue of lactose carrying transgalactosylations reactions, which are frequently used for the synthesis of galacto-oligosaccharides (GOS), attractive prebiotics (1). Metagenomics has contributed to the exploration of heated habitats such as thermal springs, either for ecological study or for bioprospection of novel enzymes. Two thermal enzymes with β-galactosidase activity have been previously isolated from hot springs using functional metagenomics (2,3). In the present study, a plasmid metagenomic DNA library was constructed from As Burgas hot spring water (Ourense, Galicia, Spain), and a novel thermostable β-galactosidase has been isolated and characterized through functional screening of the metagenomic library.

## 2. MAIN GOAL

The main objective of this work was to construct a plasmid metagenomic library to discover and characterize novel thermostable β-galactosidases from As Burgas hot spring water.

## 3. RESULTS AND DISCUSION

The search for these new thermoenzymes was carried out by construction and screening of a plasmid metagenomic library from As Burgas thermal spring water samples (Figure 1).

### *1) Isolation, DNA extraction and metagenomic library construction*

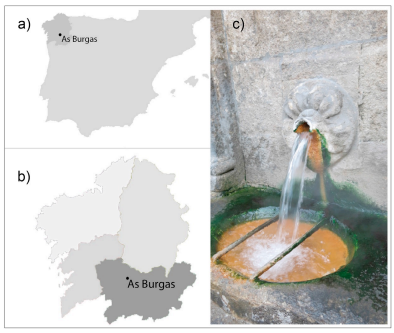
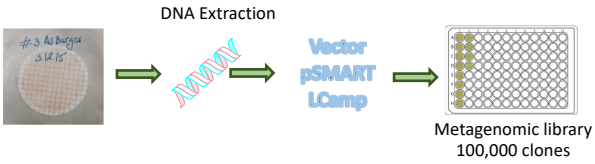



**Figure 1.** Geographical location of As Burgas hot spring in Spain (a) and in the Galician region of Ourense (b). Water spout from As Burgas, where samples were taken (c).




### *2) Search for β-galactosidases in the metagenomic library*

Functional screening for β-galactosidase activity was done by blue colonies isolation on LB agar supplemented with 100 µg mL-1 ampicillin and 0.04 % X-Gal (5-bromo-4-chloro-indolyl-β-D- galactopyranoside) in N,N-dimethylformamide.

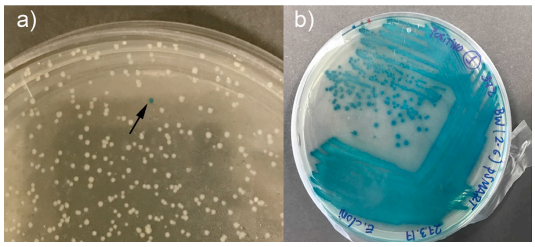
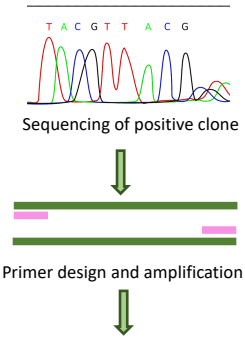



**Figure 2.** Activity screening of the metagenomic library. a) β-galactosidase positive clone (marked with an arrow). b) Isolation of the positive clone

### *3) Expression and purification of β-galactosidase*



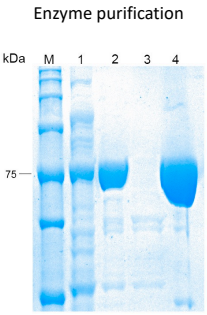




**Figure 3.** Coomassie blue-stained SDS-PAGE profile of recombinant β-galactosidase purification. Lanes M, protein marker, 1. Supernatant of E.coli T7 Express C2566 cells lysate, 2. Supernatant after heat purification at 70 °C, 3. Flow through after passing the Ni-sepharose resin, 4. Purified recombinant His tagged-β-galactosidase.

### *4) Biochemical characterization of the enzyme*

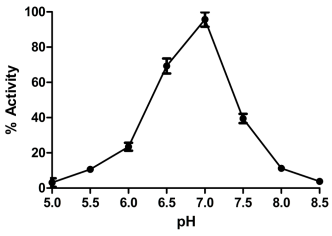



**Figure 4.** Effect of pH on the activity of purified enzyme.

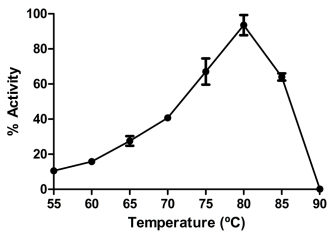



**Figure 5.** Effect of temperature on the activity of purified enzyme.

## 4. CONCLUSIONS

A novel thermostable β-galactosidase has been discovered through functional screening of a plasmid metagenomic library constructed with DNA from As Burgas hot spring (Ourense, Spain). This is the first reported thermostable β-galactosidase from family GH35 and showed an optimal temperature at 80 °C, higher than the reported for other thermostable β-galactosidases. The enzyme developed maximal activity at pH 7, near to the natural milk pH, revealing its suitability for its use in the dairy industry for the generation of low-lactose products.

## 5. FUTURE PERSPECTIVES

As the substrate specificity assays revealed that the enzyme was also able to hidrolyze p-Nitrophenyl-β-D-fucopyranoside, studies are being conducted to detect fucosyltransferase activity that could be used for the synthesis of fucosylated oligosaccharides (FUCOS) with biological interest.

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