# Hydrothermal vent biodiversity holds enzymes valuable for biotechnology

## **1. Introduction**

The aim of this study is to express and purify clostripain using Escherichia coli pLysS cells. Clostripain is a family of hydrolytic enzymes found in the metagenome of archaea living in hydrothermal vent communities. These enzymes are proteases, they catalyze the breakdown of other proteins. Proteases are a highly desirable tool in different industries, for example food, detergent and pharmaceutical industries.

#### 1.1. Archaea sequence

The sequence was obtained from a metagenome-assembled genome (MAG) with the following taxonomy: d\_Archaea;p\_\_Thermoplasmatoata;c\_\_E2;o\_\_U BA202;f JdFR-43;g ;s



Figure 1: In situ incubation in the Jan Mayen hydrothermal Vent Field on the Arctic Mid-Ocean Ridge.



S Figure 3: Solubility test showing soluble (S) and total (T) protein. Red square targets molecular weight corresponding to clostripain.



In situ incuba Hydrotherm vents Expression Incubation i media at 3

## 2. Overview of methods

ion al	$\rightarrow$	Transformation Heat shock in 42°C water bath	$\rightarrow$	Culture Agar plates with ampicillin
_B C	$\rightarrow$	Breakdown Lysis buffer and ultrasonication	$\rightarrow$	Purification IMAC on Äkta Start HisaTrap

## 3. Results

IMAC purification shows high flowthrough (figure 2). Solubility test indicate low concentration of soluble protein (figure 3). The protein is a signal peptide, peptidase C11, with 412 amino acids and a molecular weight of 47.8kDa (figure 4).

### 4. Discussion

The fractions from the IMAC purification are very small, meaning that clostripain didn't bind to the ligands in the column. This can also be observed in the solubility test, a confirmation of little soluble protein. A reason for this can be that E. coli produces the proteins too rapidly, not giving the protein time to fold correctly, leading to aggregation with itself and other proteins, making it insoluble.

Clostripain was therefore not sufficiently purified. Doing it again using different E. coli cells could improve the result, specifically BL21-Gold cells.





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