Recombinant production of enzymes from deep-sea hydrothermal vent habitats.

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BACKGROUND

Thermophilic microorganisms thrive in extreme environments like the hydrothermal vents. The microbial, functional and biochemical **diversity** in these habitats remain mostly unknown. Studying enzymes from microorganisms of such environments can teach us a about their biology and metabolism.

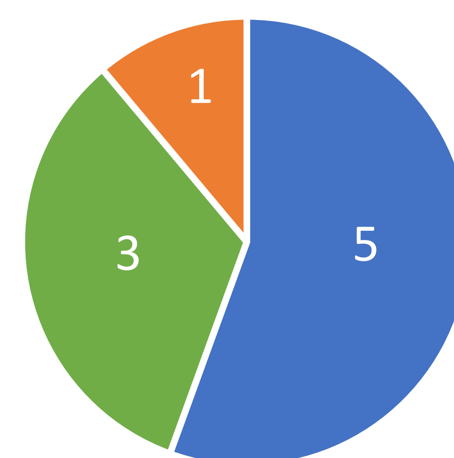
Extremophile organisms have unique adaptations based on their niches. These traits can come in the form of thermostable enzymes found in thermophilic hosts and can be valuable assets for biotechnology and research, such as proteases and nucleases which are protein and DNA degrading enzymes, respectively.

METHODS

A large number of tools and methods were used during this project including various bioinformatic tools like HMMER[1] and Blast[2]. Methods used include molecular transformation, recombinant expression and affinity purification protocols, and a large variety of instruments like centrifuges, incubator, sonicator, gel electrophoresis.

ENZYME CANDIDATES

- Protease
- Nuclease
- Polymerase



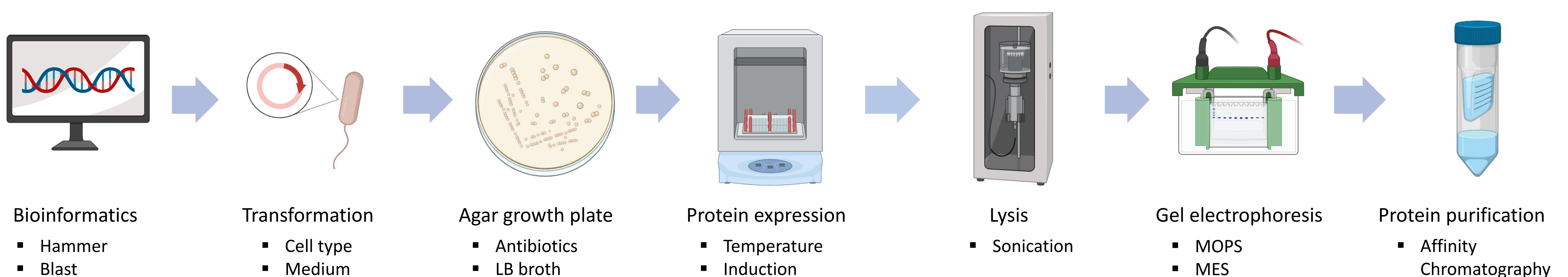
WHAT AND WHY

The aim was to produce a variety of putative enzymes in the lab with the use of recombinant protein expression in *E. coli*, including proteases, nucleases and DNA polymerases.

The candidates are from various hydrothermal sites, and are selected to hopefully show desirable traits, such as thermostability.

Nine enzyme candidates, **five** proteases, **three** nucleases and **one** polymerase. These enzymes are the focal point of this project.

PROJECT PIPELINE



RESULTS

A total of six round of expression experiments were carried out where 82 fractions were tested in total; yielding one strong and three weak soluble fractions observed from two enzyme candidates. Even after changing parameters like amount of IPTG inducer, size of growth culture, temperature, and cell type, some candidates still didn't appear soluble after recombinant expression.

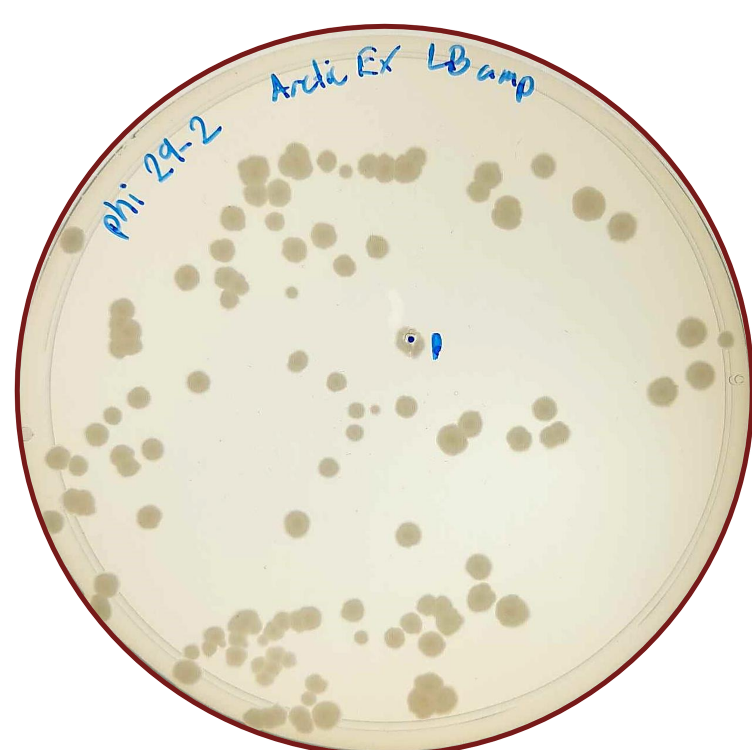


Figure 1. *A Lysogeny broth agar plate with Phi 29-2 polymerase transformed into ArcticExpress (DE3) Competent Cells. The gray spots are the E.coli colonies and the colony marked with a dot and a number one is the colony that was chosen and used for the experiment.*

SDS Page results (Total vs. Soluble)

Sequence name:	T	S
Clos 4-1a		
Clos 5-2a		
Gin 4		
Gin 26		
Lam 10		

Figure 3. *Overview of results from a gel, where the soluble (S) and total (T) samples corollate their respected candidates shown in Figure 2. The color of the cells represent the amount of protein expressed. Where dark green is strong, light green is weak, yellow is extremely low and red is no expression.*

Lam 10 and Clos 4 where the only samples that had expressed a soluble enzyme. Where Lam 10 had strong expression and Clos 4 expressed a soluble enzyme to some extent as shown is figure 2 and 3.

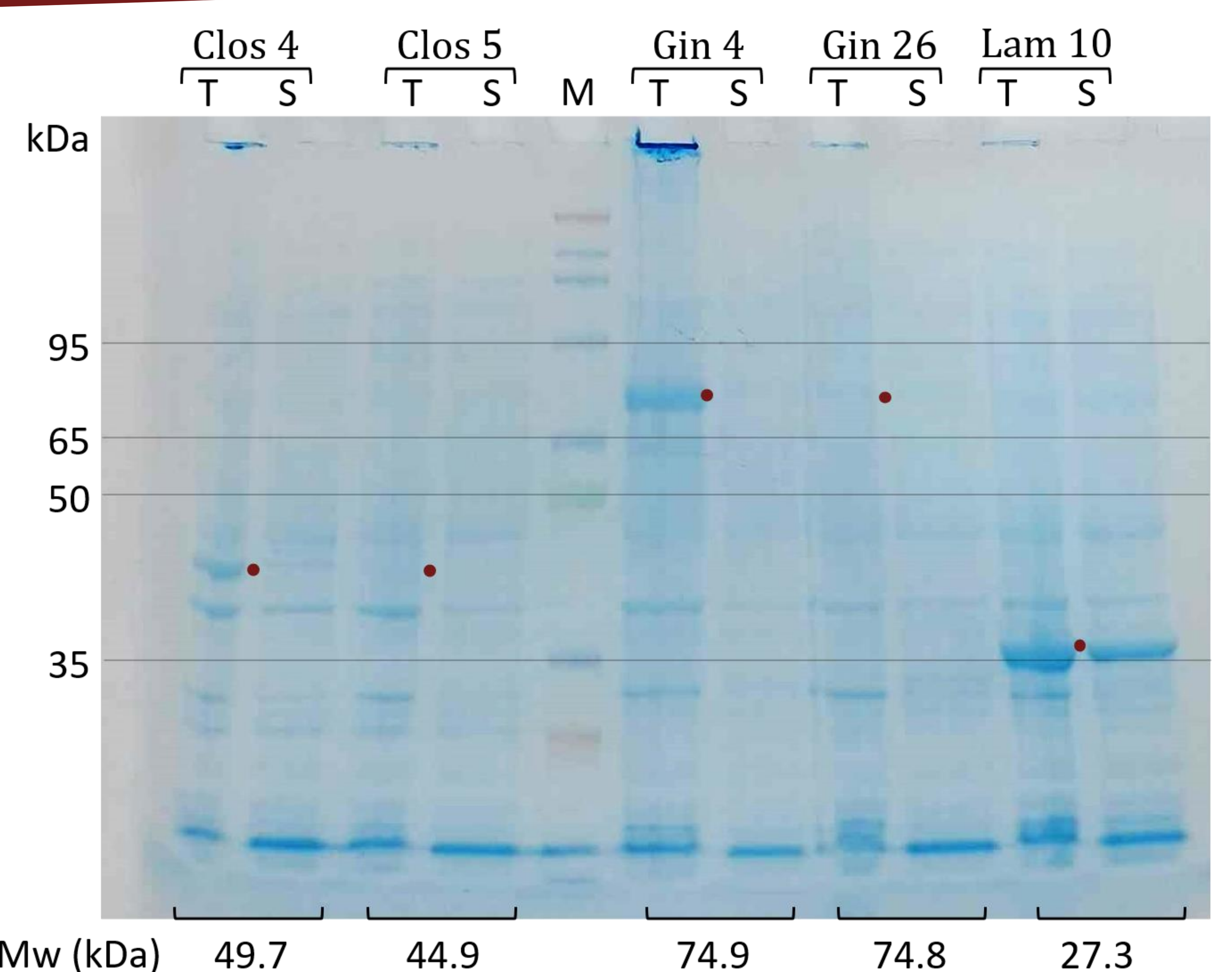


Figure 2. *SDS-PAGE showing the expression of five of the enzyme candidates. The top X-axis shows the candidates and the different samples for total lysate sample (T) and the soluble lysate sample (S). The y-axis shows the gel ladder[3] (kDa) and bottom X-axis shows the molecular weight of the enzyme (kDa). The different bands show how much of the enzymes which was produced, and the red dots show the expected position of the expressed enzyme.*

WHAT NEXT?

Soluble candidates Clos 4 and Lam 10 have been taken for further characterization after the conclusion of this project. Further work can still be done to improve the solubility of the other candidates by further experimenting with conditions, such as changing the amount of IPTG, expression temperature to control the expression speed or using different pH in the buffers for lysis and purification.

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