Can we use bacteria to clean up our mess?
A Fervidobacterium pennivorans strain degrades feathers

Tana-Helén Meyer-Becker, Rubén Javier Lopez, Nils Kåre Birkeland
Department of Biological Sciences, University of Bergen, PO box 7803, 5020 Bergen

1. Introduction

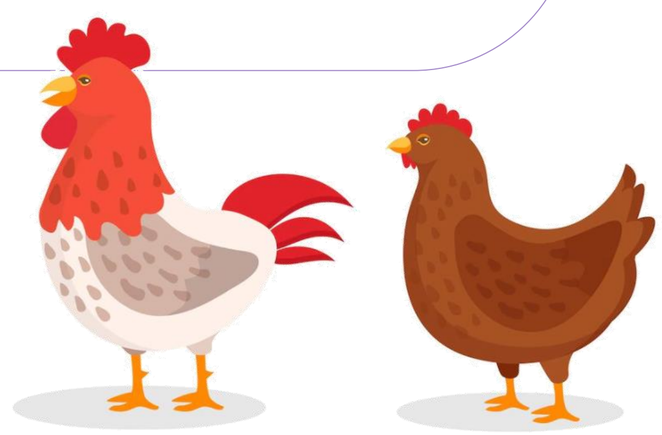
Feathers from poultry food production are frequently discarded, burned, or turned into feather meals. Feather meals are degraded feathers that are fed to animals, such as fish. They are frequently rejected due to their low quality and indigestibility [2].

Feathers contain 90% keratin, and *Fervidobacterium pennivorans* degrades keratin into a fine powder that can be used to make feather meals [2]. However, we don't know how the process works. *F. pennivorans* is an anaerobic thermophilic bacterium, that thrives best at 65 °C [1].

Two proteases that are expressed more strongly when *F. pennivorans* degrades feathers, is an M42 family metallopeptidase and a prepilin peptidase [3]. It's uncertain what role they play in feather degradation. Testing these proteases provides more information about how the thermophilic bacteria function, and how we can use it in the future.

2. Methods

- Amplification of the genes coding for the proteases by PCR
- Transformation of synthetic genes in an expression vector to BL21 *Escherichia coli*
- Heterologous protein expression
- SDS-page gel
- Purification of the protein



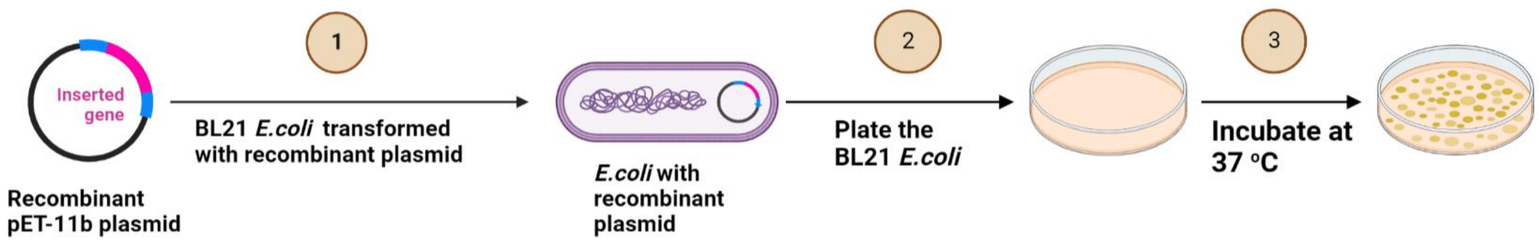


Figure 1: Transformation. 1) Synthetic genes of the proteases inside an expression vector was transformed with BL21 *E.coli.* 2 and 3) The *E.coli* bacteria with recombinant plasmid was plated and incubated at 37 °C.

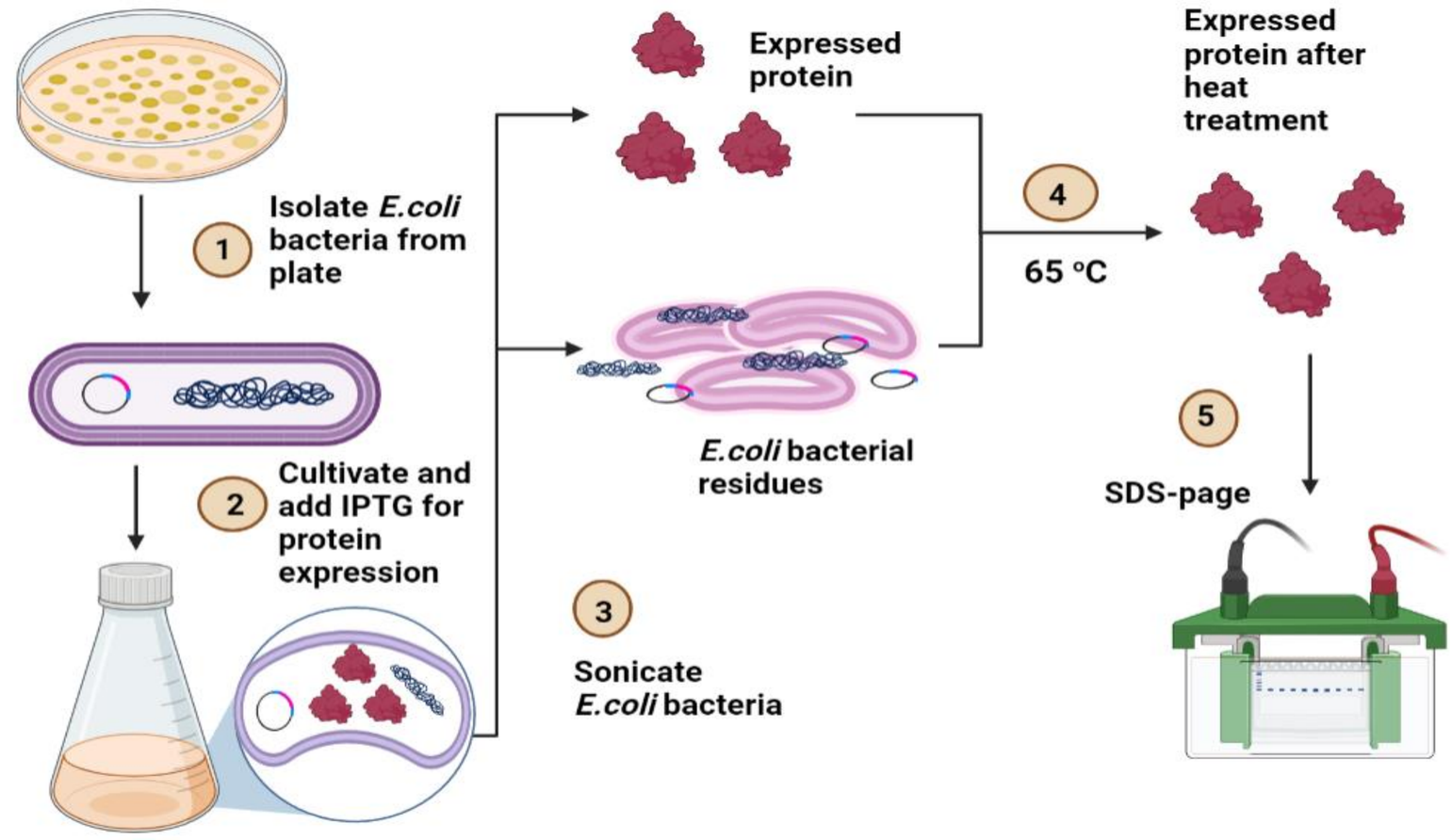


Figure 2: Protein expression. 1) Isolate *E.coli* transformed bacteria. 2) Transfer *E.coli* into falcon tube with medium for growth, express the proteases protein with IPTG. 3) Lysozyme is added, and sonication is preformed to destroy the BL21 *E.coli* cells. 4) The sample is heat treated in 65 °C to get rid of bacteria residue and non thermophilic proteins from the *E.coli. 5)* Run the proteins in an SDS-page.

3. Key Results and Conclutions

- The M42 family metallopeptidase, 3445, is expressed as a 37kDa protein, but seems to be insoluble.
- Prepilin peptidase, 4485, is poorly expressed as a 27kDa protein.
- 4885 is most likely toxic since the expression of the proteins is low.
- Even though 4885 seems to be active, since a large amount of degraded proteins are observed in cells where it is expressed, more research is needed to determine the role of the proteases.

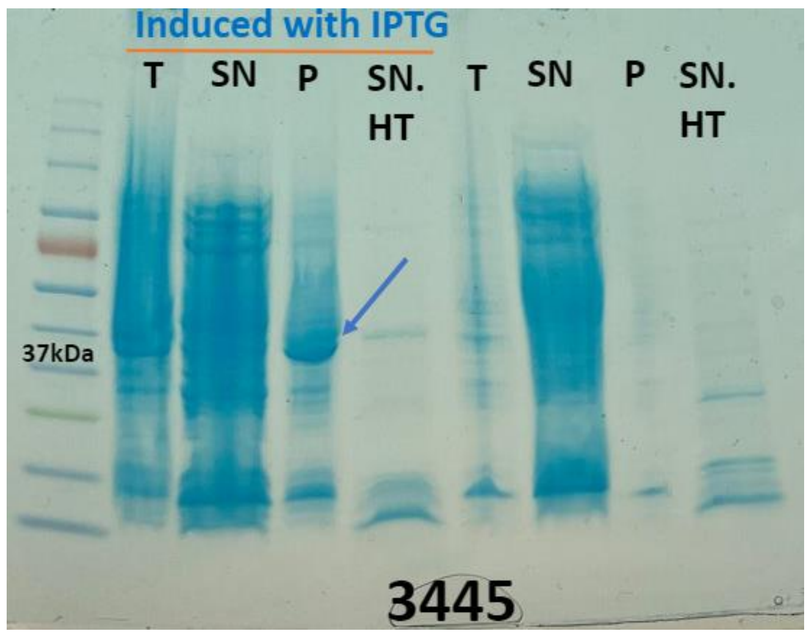


Figure 3: SDS-page of M42 family metallpeptidase. The first well is the ladder from 250-10 kDa. T is the culture, SN is the supernatant, P is the pellet, SN.HT is the supernatant heat treated at 65 °C. The first four samples are induced with IPTG, the four last samples are without IPTG. The blue arrow points to the insoluble protein.

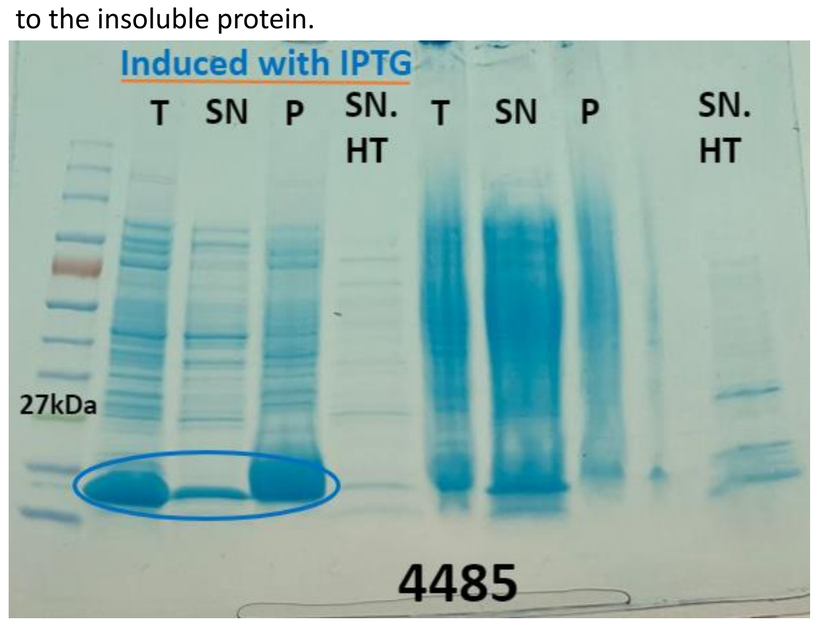


Figure 4: SDS-page of prepilin peptidase. The first well is the ladder from 250-10 kDa. T is the culture, SN is the supernatant, P is the pellet, SN.HT is the supernatant heat treated at 65 °C. The first four samples are induced with IPTG, the four last samples are without IPTG. The blue circle shows the large amount of degraded protein.

References:

1. López, R. J. (2018) 'Isolation and characterization of a new keratinolytic *Fervidobacterium pennivorans* strain from a hot spring in Tajikistan', Master Thesis, University og Bergen.
2. Of, S. and Art, T. H. E. (2019) 'FOSC Call 2019 addendum', 1(3), pp. 1–20.
3. Rubén J. López personal communication

Poster nr. 299_ 3

UNIVERSITY OF BERGEN

Extremophiles and Biotechnology research group

Tana –Helén Meyer-Becker
tme038@uib.no

University of Bergen
Molecular biology bachelor
BIO299, autumn 2022