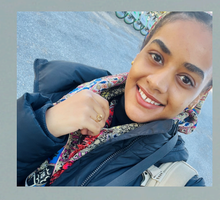
TOXIC OR NUTRIENT?



Expression and purification of a putative cyanide hydrolase enzyme

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Introduction and Background

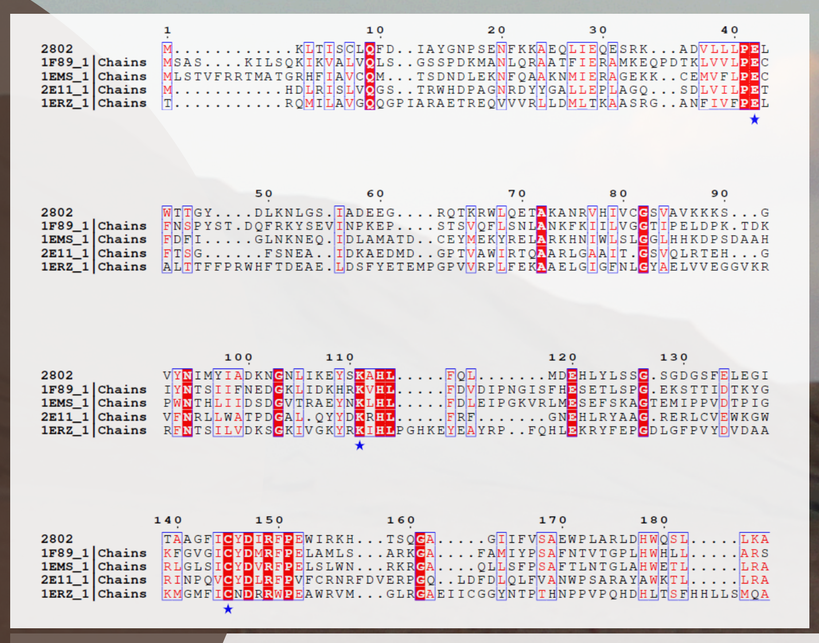
Cyanide, a lethal compound, inhibits oxygen use in mitochondria and has become an increasing environmental pollutant due to industrial activities, affecting air, water, and soil. Cyanide removal can be achieved through chemical and biological methods, with **bioremediation** being an **economical and environmentally friendly alternative** [1-4]**. Our project aims to extract and characterize a putative cyanide hydrolase enzyme from *Bacillus haynesii* NRRL B-41327, a strain well-adapted to cyanide contamination, isolated from a cyanide-polluted mining site in northeastern Sudan.**

Procedures and Outcomes

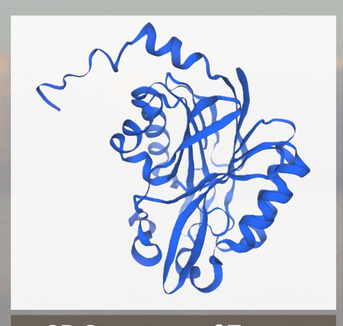
We **successfully expressed** and purified the target protein using the following steps:

1 Exploring Protein Sequences

We **extracted** DNA, **sequenced** it with Illumina, **assembled** using CLC Genomics Workbench, and **identified the gene/protein sequence** with RAST. Sequence **alignment** was performed with MAFT, the **active site was identified** using ESPript 3.0, and the enzyme was **modeled** with Swiss-Model.



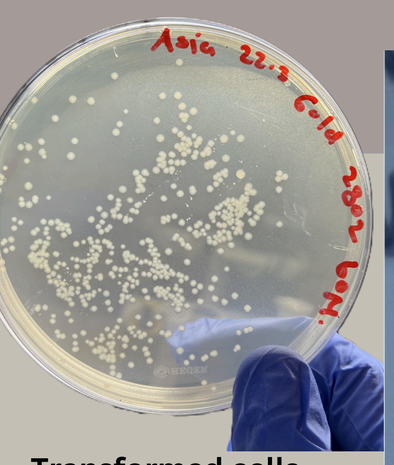
Multiple sequence alignment was conducted on proteins from our ***Bacillus haynesii* NRRL B-41327 strain**, comparing them with proteins from species such as *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Xanthomonas campestris*, and *Agrobacterium sp.* All sequences, **sourced from PDB,** were aligned using MAFFT and analyzed with ESPript 3.0. **Conserved regions and active sites**, highlighted by a **blue star at positions 42, 111, and 145**, are referenced by Chin et al. (2007) and Tsai et al. (2006).



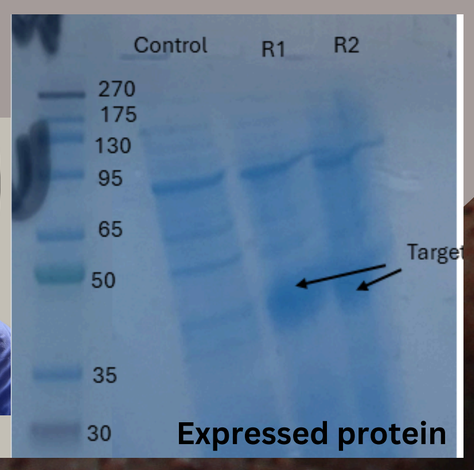
3D Structure of Target Protein via Swiss-Model

2 *Expressing the Protein*

A synthetic gene including an N-terminal **His-Tag** was made in vector pET-28b and **codon optimized for *E. coli*** using GeneScript, then **transformed** into BL21-Gold(DE3) competent cells, with protein **expression** subsequently **induced** by **IPTG**.



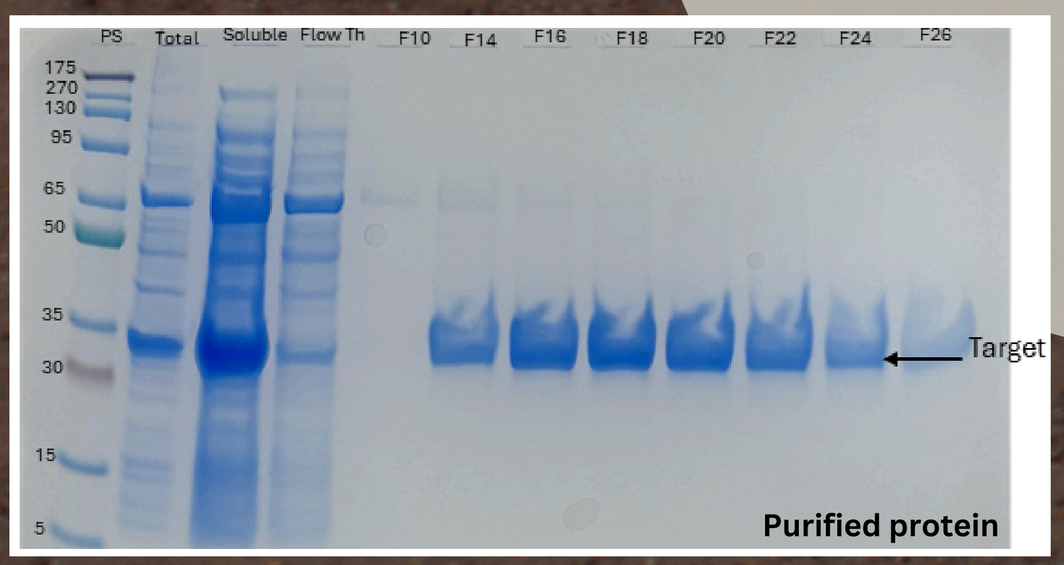
Transformed cells



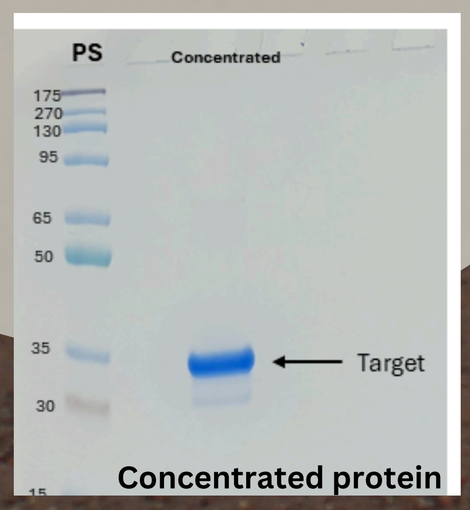
Expressed protein

3 Purification of the Expressed Protein

The protein was **purified** using IMAC with an ÄKTA Start system and a 5 ml HisTrap FF Column, then **concentrated** and underwent **buffer exchange** from imidazole to Tris-HCl EDTA using an Amicon® Ultra-15 30K.



Purified protein



Concentrated protein

Discussion and Conclusion

Our findings **align** with the results from Chin et al. (2007) and Tsai et al. (2006), **suggesting that our protein is a member of the CN-hydrolase superfamily.** We believe we have successfully expressed the target protein and identified it as a putative cyanide hydrolase enzyme. However, **we must now confirm its activity through laboratory tests.**

References

1: Jaszczak et al., 2017, 2: Nallapan Maniyam et al., 2013.
3: Akcil, 2003, 4: Tiong et al., 2014.

Acknowledgment

PN: 299_3

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