Heterologous expression and characterisation of novel chitinases from a yet unculturable, highly abundant bacterial symbiont of octocorals

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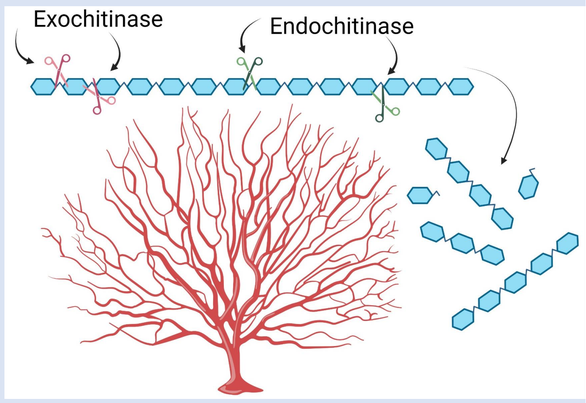
Introduction



- Chitin is the most abundant natural polymer in marine settings.
- Octocorals (p: Cnidaria) are suspension-feeding marine invertebrates that consume a chitin-rich diet (e.g., zooplankton, diatoms).
- Their microbiomes are likely well adapted to chitinous food processing.
- The marine bacterial family *Endozoicomonadaceae* is an important part of the coral microbiome, reaching high relative abundances and is an indicator of coral health **[1]**.

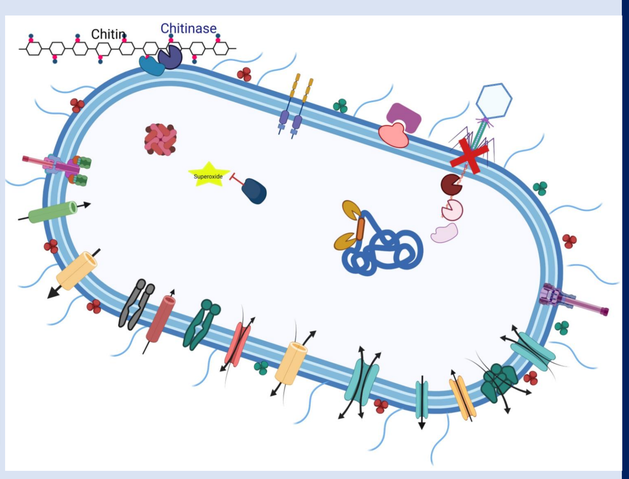
Endozoicomonadaceae symbionts of corals

Involved in amino acid and vitamin B supply to the host, micronutrient acquisition, sulfur cycling and carbohydrate metabolism **[2]**.

Only a few species are successfully cultured and maintained in the laboratory, severely limiting investigation of their ecology & physiology.

A novel, yet uncultured genus, *Candidatus* Gorgonimonas, from the Endozoicomonadaceae family was proposed by our team in 2022 **[2]**.

A metagenomics survey showed that *Candidatus* Gorgonimonas harbours endo-chitinase genes with low homology to known chitinases (< 50%) **[3]**.



[1] Keller-Costa et al., 2021, Microbiome, 9, 1-21 https://doi.org/10.1186/s40168-021-01031-y
[2] Keller-Costa et al., 2022, Microbiome 10, 151 https://doi.org/10.1186/s40168-022-01343-7
[3] Silva et al., 2023, ISME Communications 3, 109 https://doi.org/10.1038/s43705-023-00316-7

Objectives

- Synthesize *de novo* the chitinase gene from uncultured octocoral symbiont *Candidatus Gorgonimonas* and express it In *E. coli.*
- Characterize the chitinase activity over a range of temperatures and pH with fluorochrome-labeled substrates.
- Analyze the substrate consumption (colloidal chitin and chitosan) and chitin oligomer production pattern of the enzyme (Ongoing!).

Metagenomics-based predictions of the chitinase:

The native gene was 1644 bp, and the native protein 576 aa (pI 4.89, 64.6 KDa).

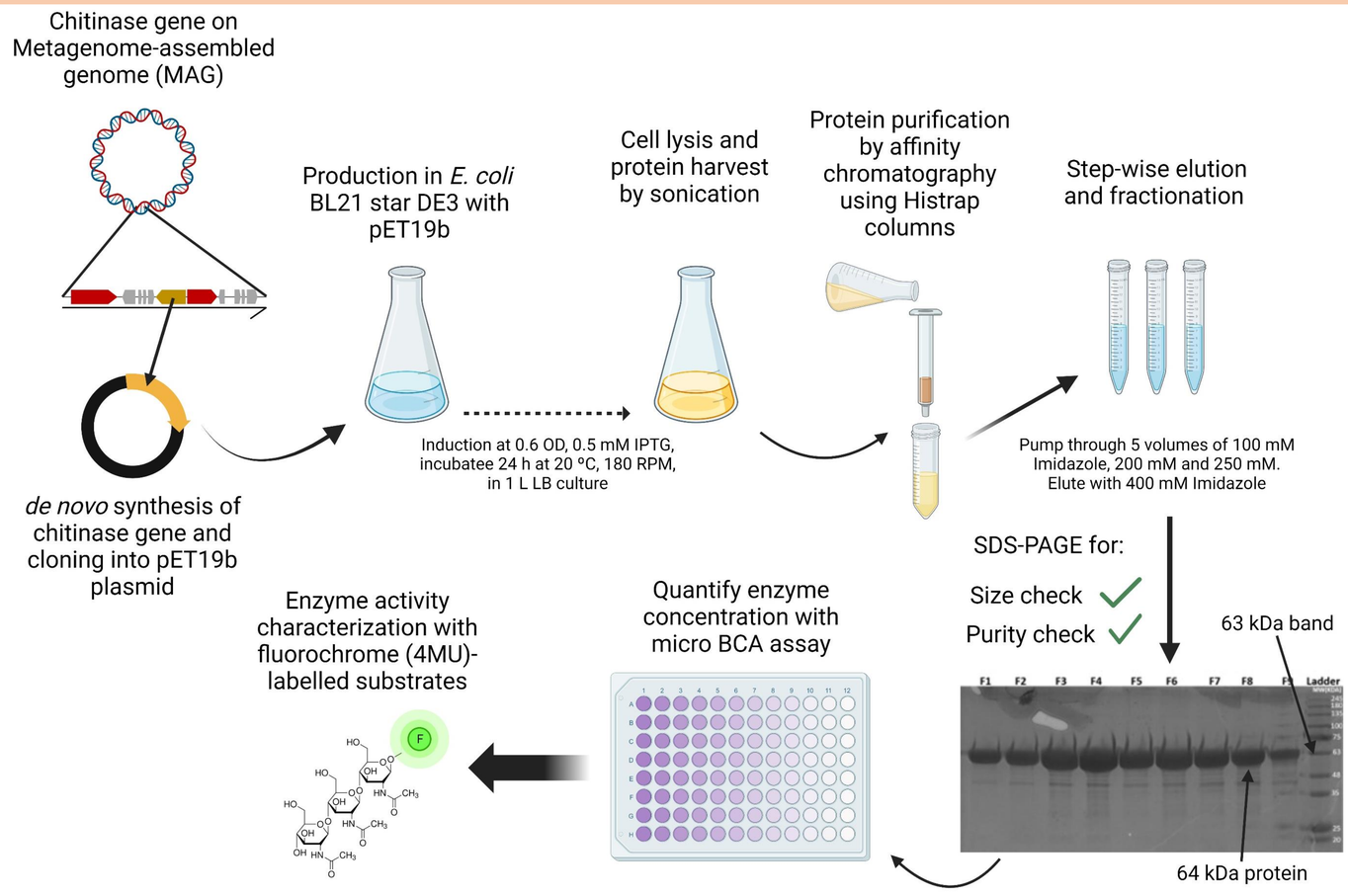
The recombinant protein (- signal peptide, + Histidine tail) is 571 aa, has a pI of 5.13 and 64.1 Kda.

The enzyme was predicted to be an endochitinase (EC 3.2.1.14), it had a GH18 protein family domain, and two unknown domains, DUF5011 and DUF5011.

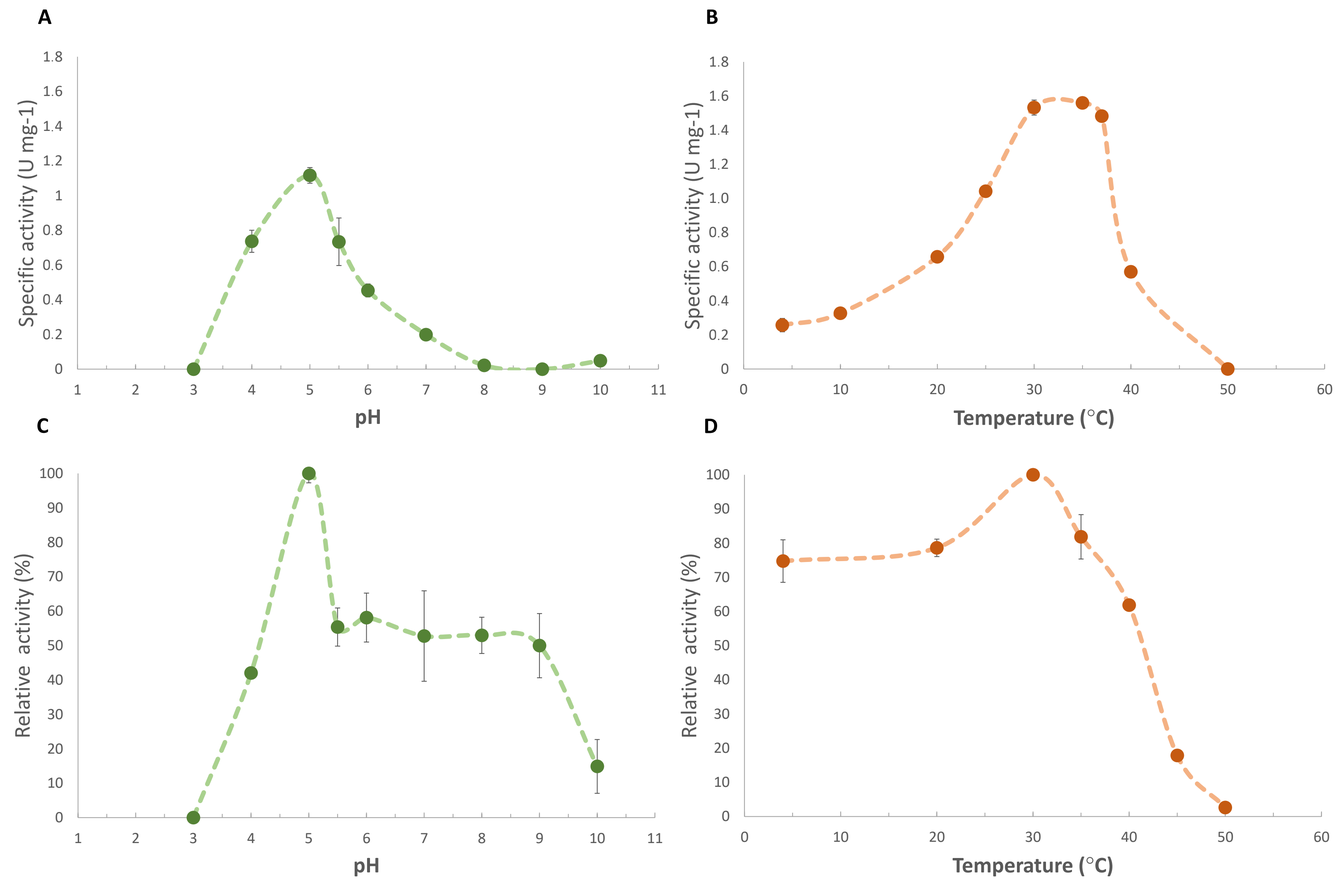
Conclusion

- The here produced recombinant protein from yet uncultured octocoral symbiont *Candidatus* Gorgonimonas presents endo-chitinase activity but no N-acetylglucosaminidase (exo-chitinase) activity.
- The endochitinase was active in the range of 4 – 40 ºC, and 4 – 7 pH, with highest activity (1.6 U mg-1) being reached at 30 – 37 ºC and pH 5.
- Although stability is highest at pH 5, the chitinase is also stable at neutral to alkaline pH (until pH 9) values and temperatures up to 40 ºC, retaining > 50 % activity.
- The chitinase presents a V_{max} of $0.48*10^{-3}$ U ml-1 and a Km-value of 0.125 mg ml-1.
- Releases chitin trimers (triacetylchitotriose) and dimers (diacetylchitobioside), but not monomers (N-acetylglucosamine).

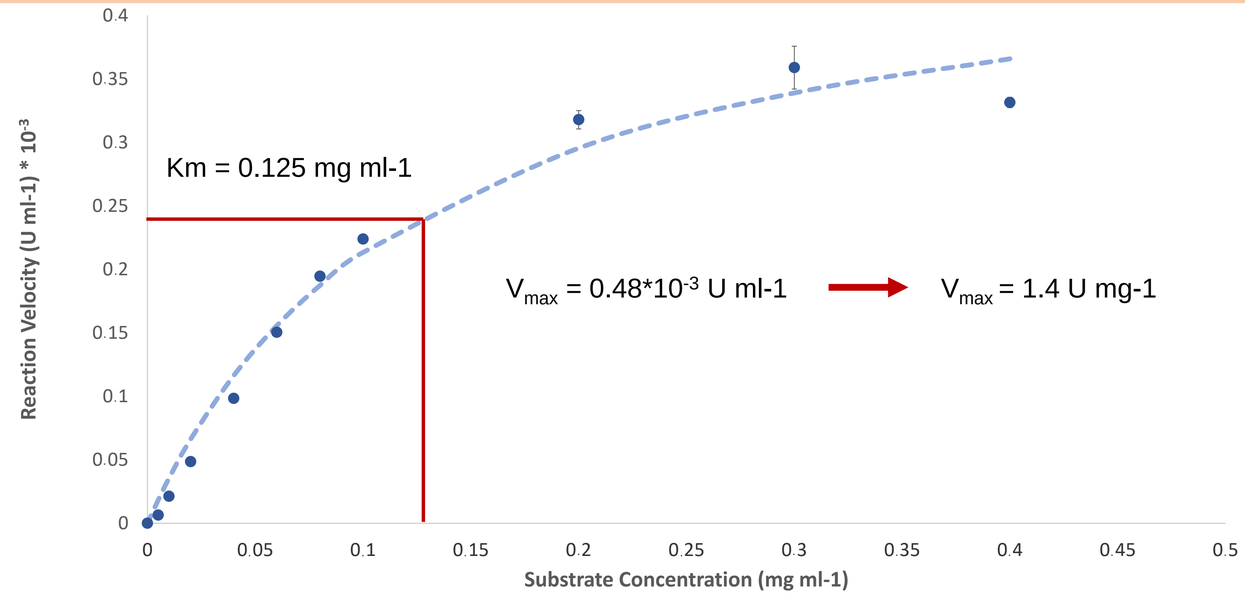
Methodology: Heterologous chitinase expression and purification



Temperature- and pH-dependent activity and stability of the chitinase



Kinetic characterization with 4-Methylumbelliferyl β-DN,N′,N″-triacetylchitotrioside



Kinetic characterization was conducted using Citric acid – Trisodium citrate 10 mM as buffer at pH 5, at 35 °C.

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