Exploiting yeast surface display for whole cell biocatalysis in *Komagataella phaffii*

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Abstract

Recombinant enzymes are usually secreted into the cultivation medium and purified at the end of fermentation, but this process can be labor-intensive and costly.

Employing whole cell biocatalysis (WCB) can reduce the number of purification steps and the catalyst can also be easily removed from the reaction mixture, facilitating recycling in industrial processes.

WCB is limited by the cell membrane, that can slowdown or prohibit substrate and product exchange: With yeast surface display (YSD), the enzyme is attached to the outer cell wall, overcoming membrane permeability limitations.

In this work, inulinase Inu1 from *Kluyveromyces marxianus* has been displayed on the surface of *Komagataella phaffii*, and its performance in terms of enzymatic activity and resistance to pH and temperature stresses has been compared to that of the free enzyme.

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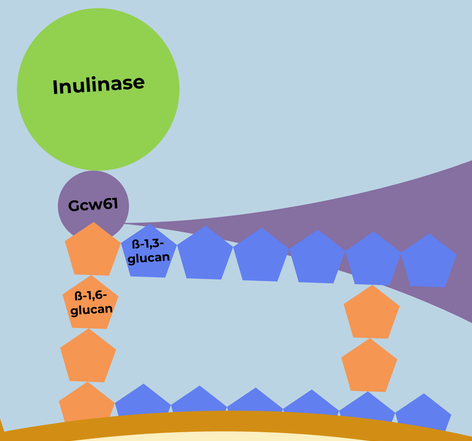
For methods and additional data



How it works

GCW61 is a GPI anchor native to *Komagataella phaffii* [1]: *when fused in frame with the protein of interest, it allows it to be covalently bound to the ß-1,6-glucans of the cell wall, displaying it on the surface*

Results

1 Growth kinetics

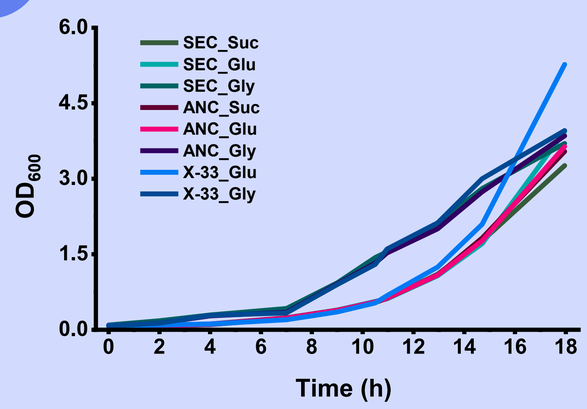


Fig. 1 Growth kinetics of the anchoring (ANC) and secretion (SEC) strains in glucose, sucrose or glycerol, compared with the wild type strain (X-33)

- No difference in growth rate between strains in the same medium: enzyme expression and display has no negative impact on cell growth
- Exponential phase slows down after OD_{600} = 3

2 Enzymatic activity

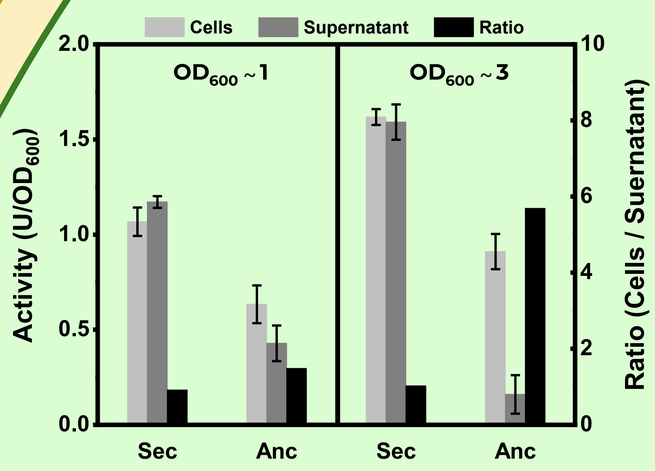


Fig. 2 Ratio and enzymatic activity in the supernatant and on cells for strains SEC and ANC, at OD600 of around 1 and 3

- The anchoring strain has a **5.6x** higher cells / supernatant ratio of activity
- Over time, activity on cells for strain ANC increases, while activity in supernatant decreases
- Strain SEC has a higher overall activity, possibly due to issues in enzyme dimerization in the ANC strain

3 Enzyme stability

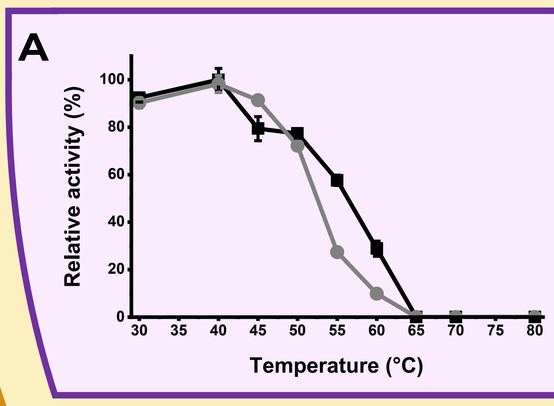


Fig. 3 A Stability of secreted and anchored enzymes after 60 minutes of incubation at different temperatures

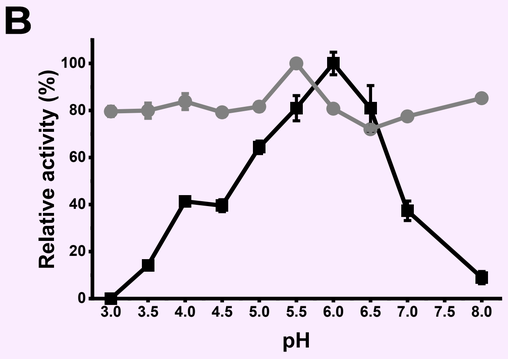


Fig. 3 B Stability of secreted and anchored enzymes after 24 hours of incubation at different pH

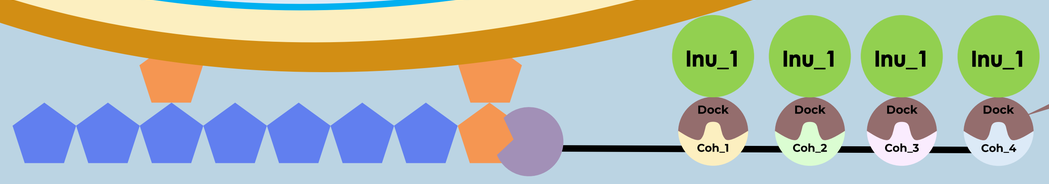
- Enzyme immobilization and surface display have been shown to increase stability to various stresses[2]
- The free enzyme has a higher stability at 55 and 60°C, but the system is more complex than it seems (check additional files if interested)
- The anchored enzyme has a much higher stability to pH

4 Conclusions

- Inulinase expression and its immobilization on the surface do not negatively affect growth, and allow *K. phaffii* to use either sucrose or inulin as sole carbon sources
- The ANC strain has a high cell / supernatant activity ratio, but something is limiting enzyme activation: further studies are needed
- Even though immobilization doesn't improve thermotolerance, the effect on pH stability is very interesting and could improve the range of enzyme utilization

Future perspectives

Taking inspiration from cellulosomes, the cohesin domains from a scaffoldin of *Clsotridium thermocellum*[3] will be expressed with the GCW61 anchor, while inulin will be expressed with a dockerin domain from the same species, with the objective of enhancing enzymatic activity associated to a single cell



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