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The Mut$^+$ strain of *Komagataella phaffii* (*Pichia pastoris*) expresses P_{AOX1} 5 and 10 times faster than Mut^s and Mut$^-$ strains: Evidence that formaldehyde or/and formate are true inducers of P_{AOX1} (1).

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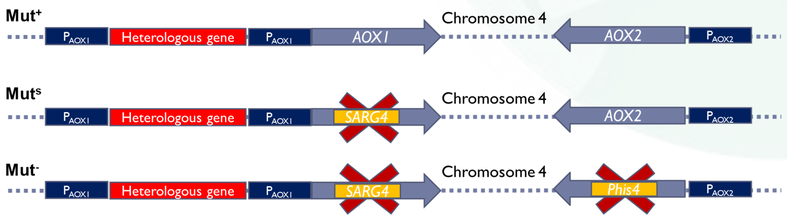
Abstract

The methylotrophic yeast *Komagataella phaffii* is among the most popular hosts for recombinant protein synthesis. Most recombinant proteins were expressed in the wild-type Mut$^+$ host strain from the methanol-inducible promoter P_{AOX1}. Since methanol metabolism has undesirable consequences, two additional host strains, Mut^s (*Δaox1*) and Mut$^-$ (*Δaox1Δaox2*), were introduced which consume less methanol and reportedly also express recombinant protein better than Mut$^+$. Both results follow from a simple model based on two widespread assumptions, namely methanol is transported by diffusion and the sole inducer of P_{AOX1}. To test this model, we studied ^{14}C-methanol uptake in the Mut$^-$ strain and β-galactosidase expression in all three strains. We confirmed that methanol is transported by diffusion, but in contrast to the reports in the literature, Mut$^+$ expressed β-galactosidase 5- and 10-fold faster than Mut^s and Mut$^-$, respectively. These results imply that methanol is not the sole inducer of P_{AOX1} - metabolites downstream of methanol also induce P_{AOX1}. We find that formate or/and formaldehyde are probably true inducers since both induce P_{AOX1} expression in Mut$^-$ which cannot synthesize intracellular methanol from formate or formaldehyde. Formate offers a promising substitute for methanol since it does not appear to suffer from the deficiencies that afflict methanol.

Introduction

- *K. phaffii* is the workhorse of Indian biotechnology industry.
- Recombinant proteins are usually produced under a strong regulatable promoter like P_{AOX1}, which is induced by methanol (2).
- *K. phaffii*, being a methylotrophic yeast, can consume methanol.
- Mut slow (Mut^s) and Mut minus (Mut$^-$) strains, with impaired methanol consumption abilities, are sometimes preferred for recombinant protein production.
- Given any recombinant protein, which host strain should we use?

Hypothesis

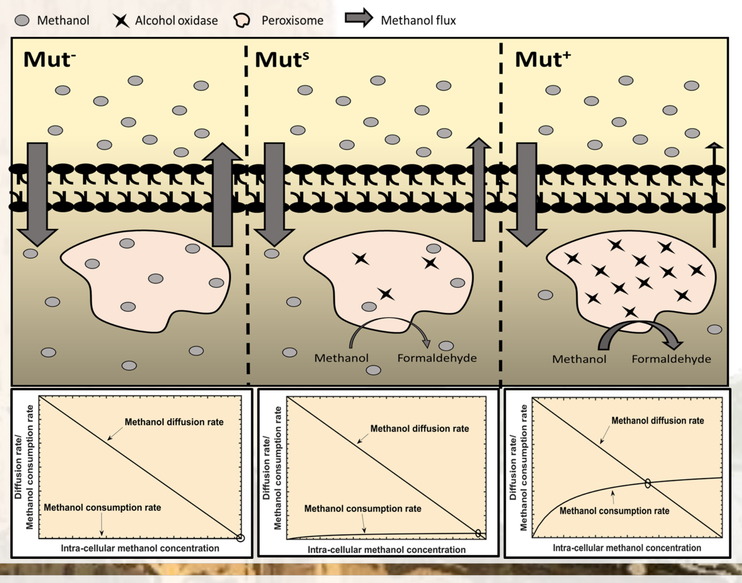


Assumptions:

I. Methanol enters the cell by diffusion rather than by active transport

II. Methanol is the sole inducer of P_{AOX1} expression

$$\frac{dm_i}{dt} = k_d(m_e - m_i) - r_m = 0$$



Methanol → Formaldehyde → Formate

Formaldehyde and Formate are key metabolites of methanol utilization pathway (3).

Conclusions

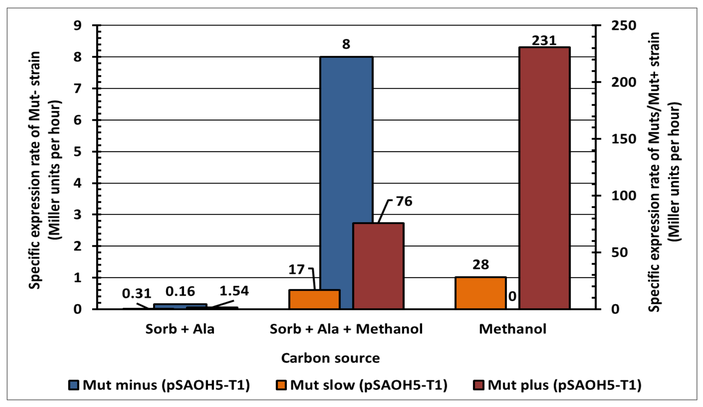
Although the AOX1 promoter of *K. phaffii* is widely used for heterologous protein production, the mechanisms regulating its expression are not well understood. For instance, it is assumed, without definitive evidence, that methanol is transported by diffusion, and is the sole inducer of P_{AOX1} expression. In this work, we demonstrated for the first time that methanol is indeed taken up by diffusion, but methanol is not the sole inducer of P_{AOX1} expression. If the latter were true, the diffusive transport of methanol implies that the Mut$^+$, Mut^s, and Mut$^-$ strains would have intracellular methanol concentrations, and hence P_{AOX1} expression rates, in the order Mut$^+$ < Mut^s < Mut$^-$, but our experiments showed exactly the opposite trend: Mut$^+$ ≫ Mut^s > Mut$^-$. We verified that the observed trend is due to differences of the AOX activities because expression (resp., deletion) of AOX in the Mut$^-$ (resp., Mut$^+$) strain transforms the P_{AOX1} expression rates to those characteristic of the Mut$^+$ (resp., Mut$^-$) strain. These results suggest that the products of AOX metabolism, namely formaldehyde or/and formate, also induce P_{AOX1} expression. We confirmed that this is indeed the case, because both formaldehyde- and formate-induced P_{AOX1} expression in the Mut$^-$ strain, which possesses no known pathway from formaldehyde and formate to methanol. We argue that formaldehyde and formate are useful alternatives to methanol, since they do not suffer from the deficiencies that afflict methanol.

Industrial Significance: True potential of Mut^s and Mut$^-$ strain can finally be realized by using formate instead of methanol

Technology Readiness level: Our group is performing studies to assess the potential of formate to induce expression of industrially relevant proteins

Results

The specific β-galactosidase activity and specific expression rate in Mut$^+$ strain is 5-10 times higher than that observed in Mut^s and Mut$^-$ strains.



$$r_{p_i}^{+} = \frac{dp_i}{dt} + r_{p_i}^{-} + r_{p_i}^{\rightarrow} + \mu p_i \quad \Rightarrow \quad r_{p_i}^{+} \approx \mu p_i$$

Expected P_{AOX1} expression rate trend: Mut$^+$ < Mut^s < Mut$^-$

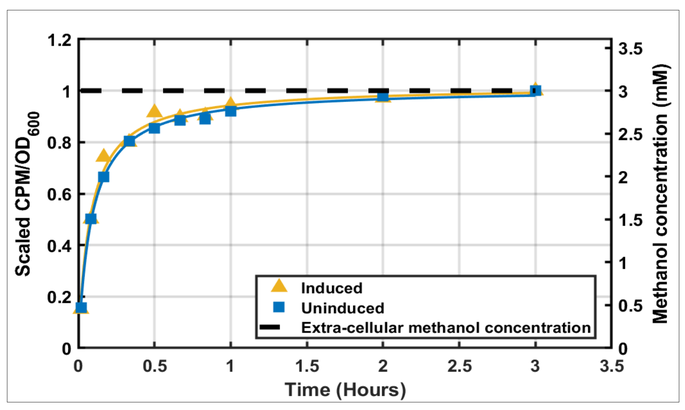
≠

Observed P_{AOX1} expression rate trend: Mut$^+$ ≫ Mut^s > Mut$^-$

Are our assumptions wrong?

$$m_i = m_e$$

(in both induced and uninduced cells)

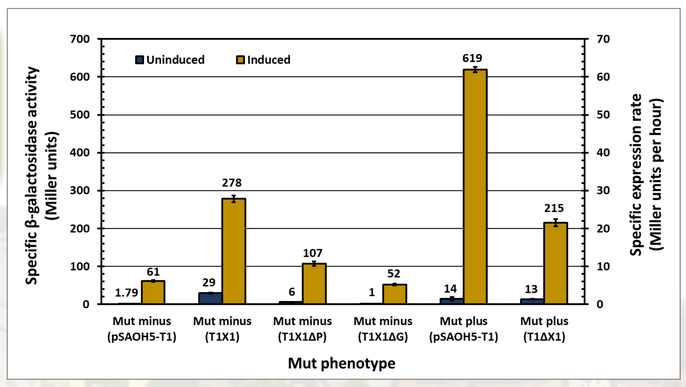


Assumption I: Methanol is transported by diffusion.

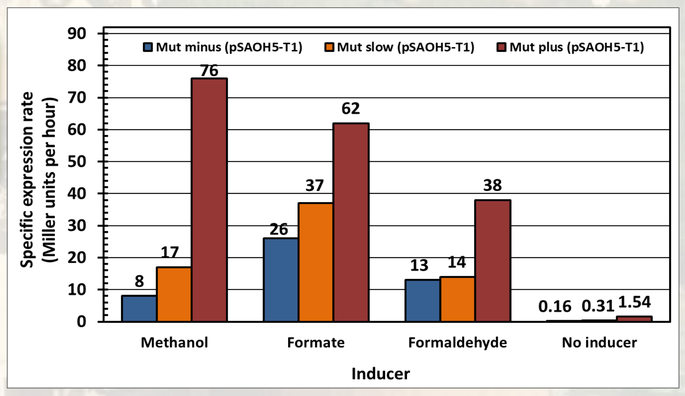
Mut$^-$ + AOX → β-gal expression↑

Mut$^+$ - AOX → β-gal expression↓

Different expression rates of Mut strains are due to differences in their AOX activities.



Formate and formaldehyde induce synthesis of alcohol oxidase



Comparison of methanol, formate and formaldehyde as inducers of protein expression in the three Mut phenotypes.

Assumption II: Methanol is true inducer of P_{AOX1}.

References

(1) Singh A, Narang A. (2019). *bioRxiv*, **1**:573519.

(2) Potvin, G et al. (2012). *Biochemical Engineering Journal*, **64:**91.

(3) De Schutter, K et al. (2009). *Nature biotechnology*, **27**:561.

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सत्यमेव जयते

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