

Improving *Aspergillus niger* as a production host through manipulation of pH responding transcription factors.

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Publication date:
2009

Document Version
Publisher's PDF, also known as Version of record

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Citation (APA):

Poulsen, L., Bruno, K. S., Thykær, J., Baker, S. E., & Eliasson Lantz, A. (2009). Improving *Aspergillus niger* as a production host through manipulation of pH responding transcription factors.. Poster session presented at Recent Advances in Fermentation Technology VIII, San Diego, California, USA, .

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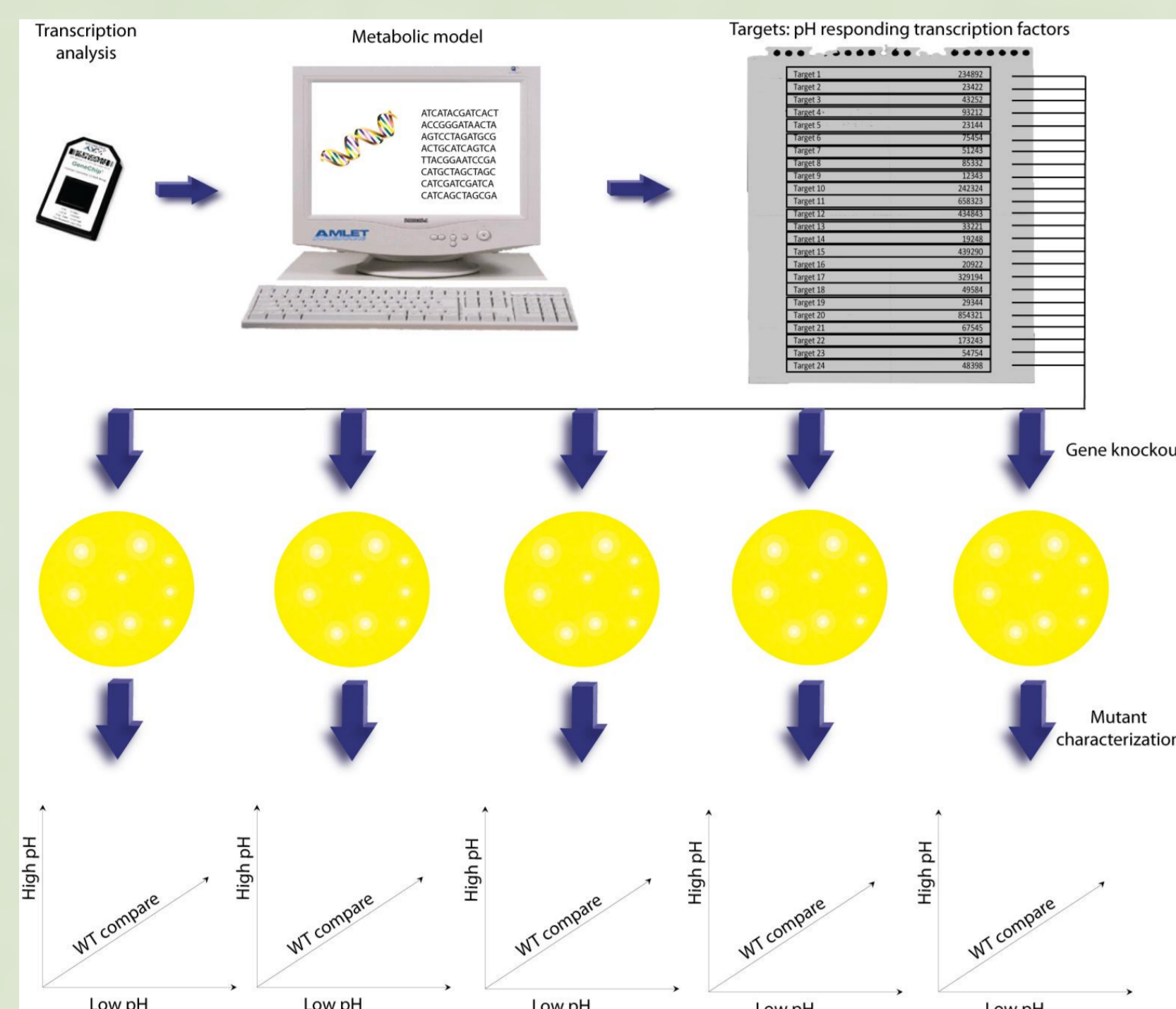
Introduction

Altering fluxes for overcoming metabolic bottlenecks have traditionally been approached by genetic engineering of a single or few metabolic genes. This strategy struggles to overcome the subsequent regulation thus the outcome has frequently shown to be of limited success. Transcription factors have the potential of controlling several fluxes in an organism, hence manipulating expression of these proteins can provide an alternative tool for overcoming metabolic bottlenecks. This approach has previously been demonstrated in yeast with great success for production of ethanol (Schuurmans et al., 2008).

In the present study the effect of modulation of transcription factors in *Aspergillus niger*, which is an industrially important micro-organism used in various processes including organic acid and enzyme production, was investigated. The strategy described in this work focuses on regulation connected to pH. It was chosen as an important process parameter, due to its significant influences on both organic acid and enzyme production.

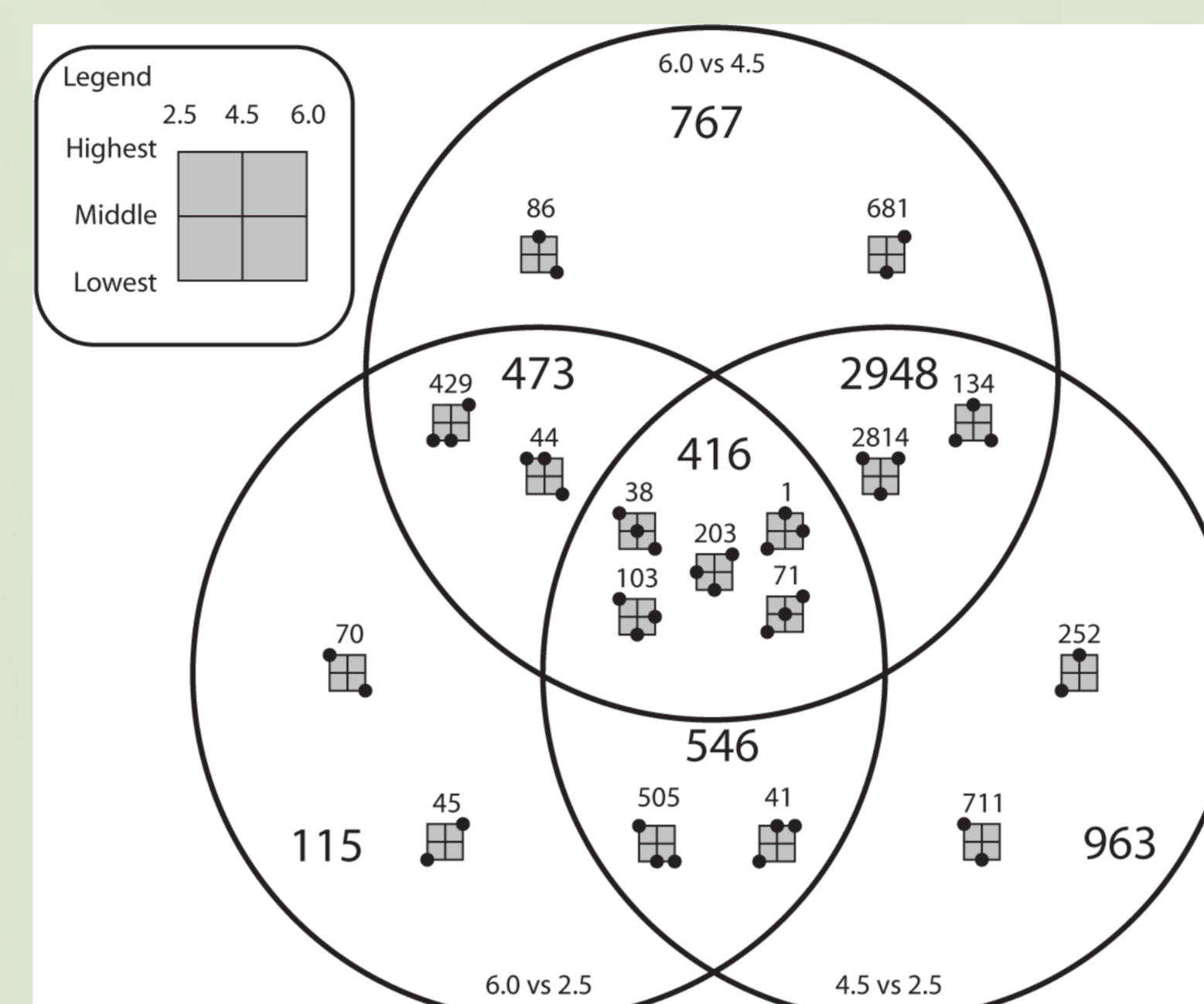
The strategy

A number of genes identified as putative pH responding transcription factors was knocked out and exposed to screening experiments. These included morphological studies (not shown), investigation of acid production and protease activity.



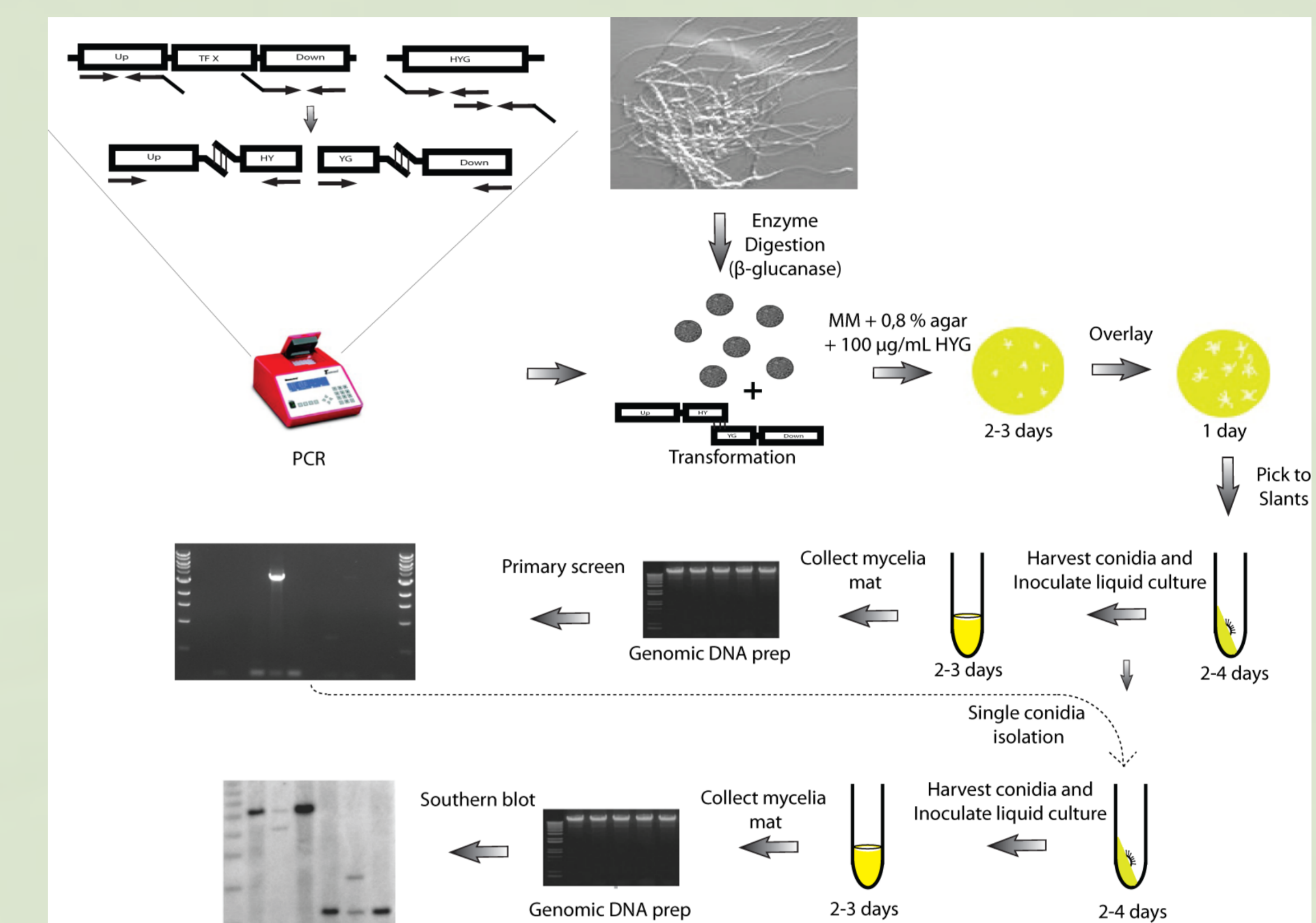
Locating targets

In order to locate targets for knockout, data from a previous transcription analysis (Andersen et al., 2009) was utilized. Several putative transcription factors with pH responding behavior was identified and formed the basis for gene-knockout.



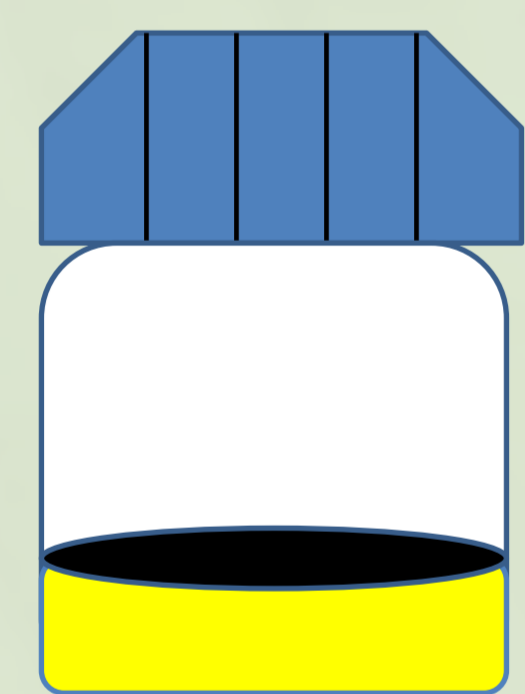
Strain construction

The *A. niger* ATCC 1015 was used as Wild-type strain and was the basis for all strain constructions.



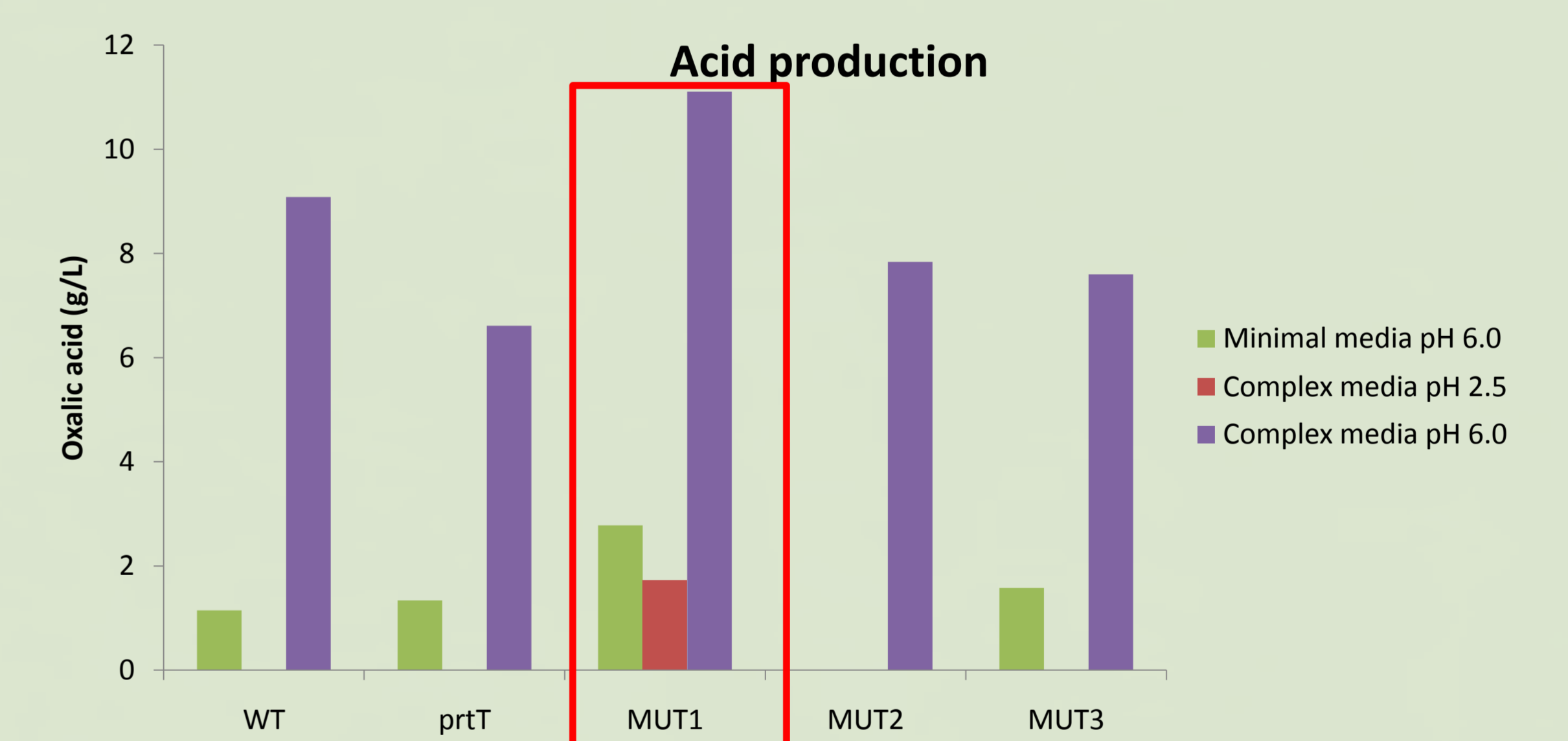
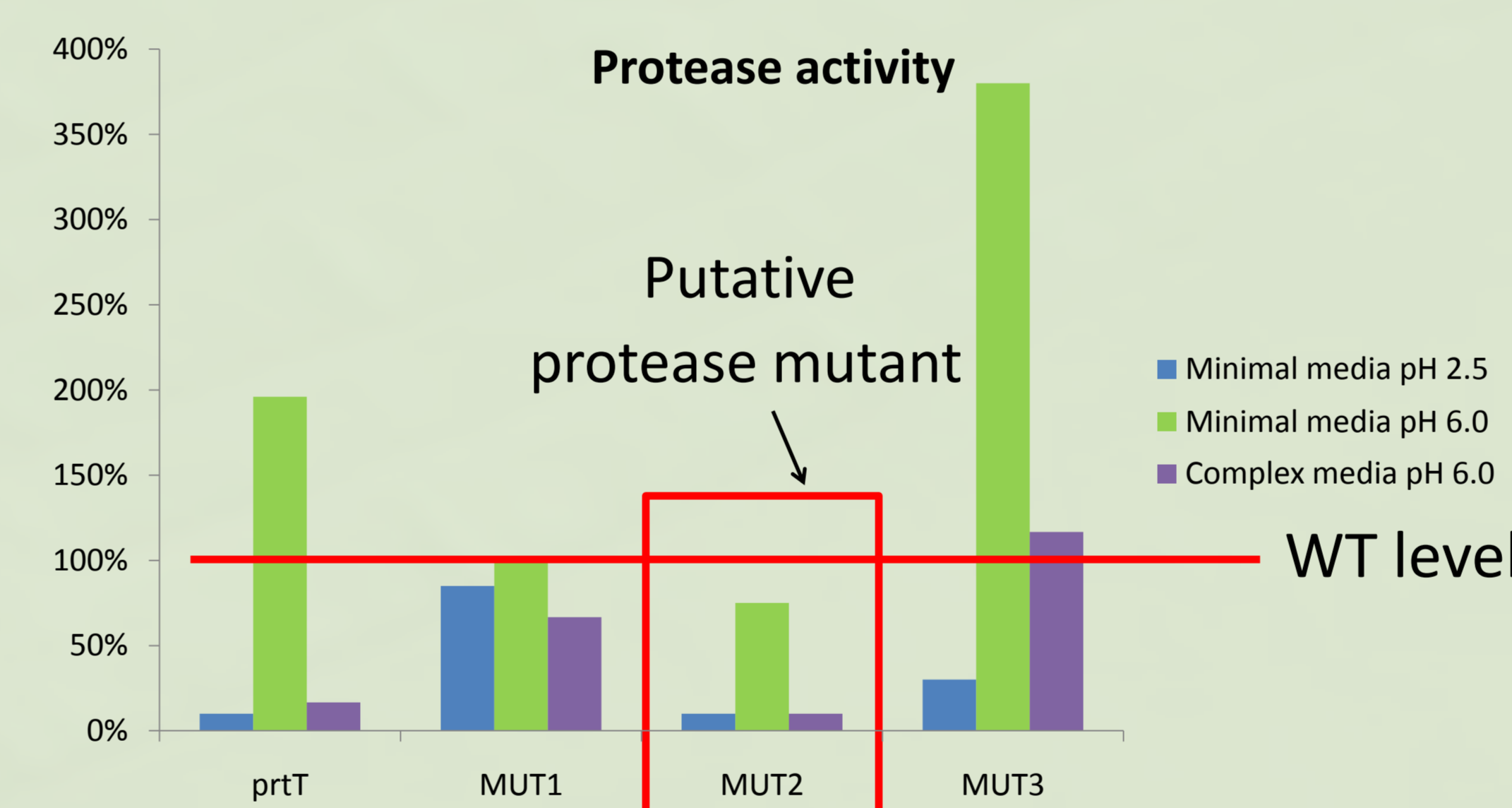
Physiological characterisation

Screening

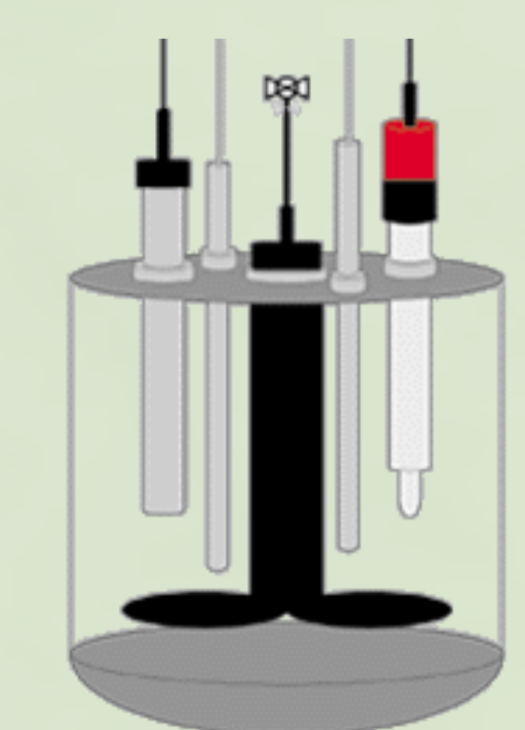


Three novel TF mutants were screened for protease activity and acid production and compared against Wild-type (ATCC 1015) and a previously described protease deficient mutant prtT (P. J Punt et al., 2008).

The experiments were performed for 4 days as 6 mL stationary cultures in minimal - and complex medium.

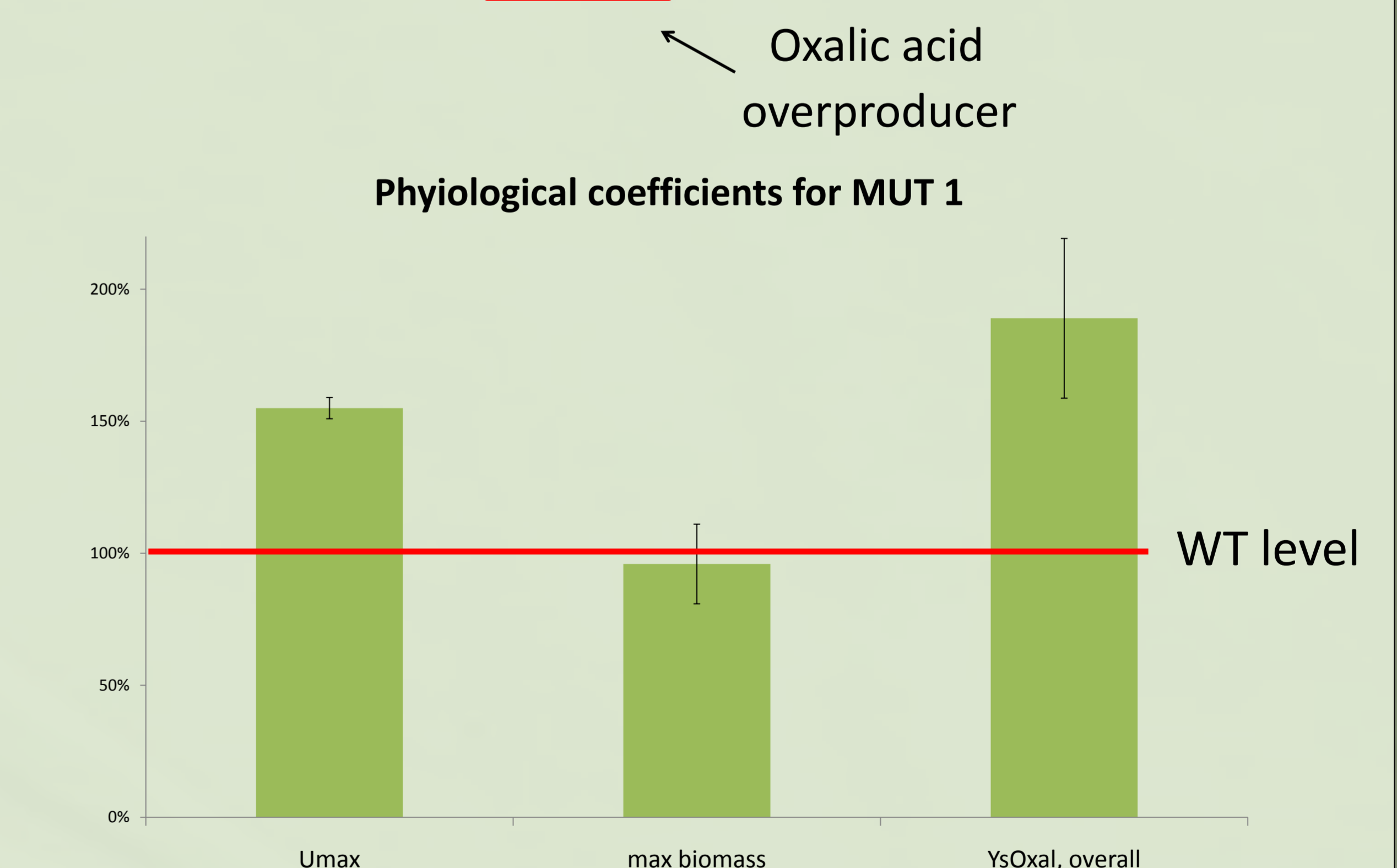
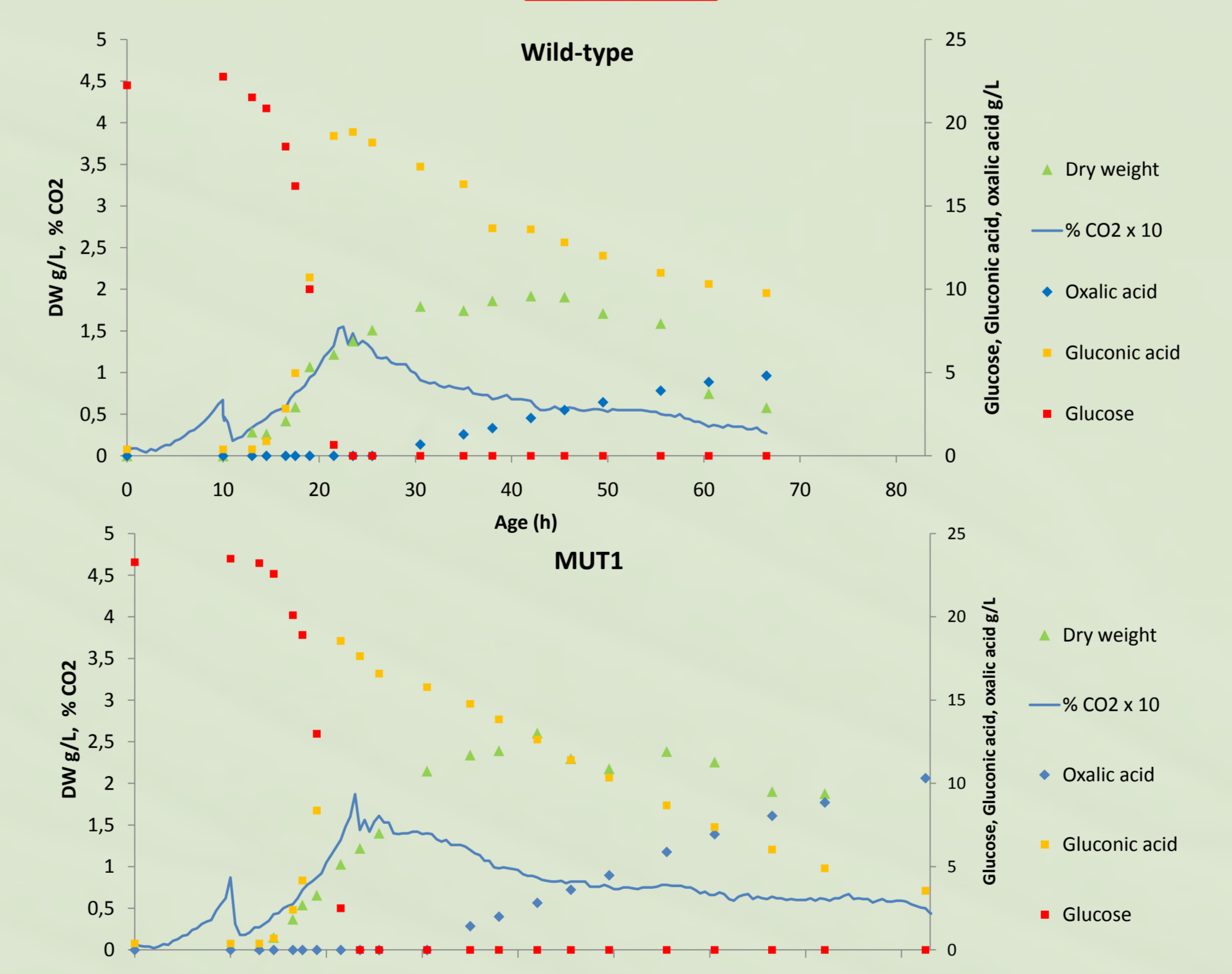


Batch fermentations



To further characterize MUT 1, which expressed an oxalic acid overproducing phenotype (OOP), this mutant was cultivated in 2L scale bioreactors.

Parameters	Germination	Fermentation
Temperature	30 °C	30 °C
Aeration	0.10 vvm	1 vvm
Stir speed	200 RPM	1000 RPM
pH	3.0	6.0



References

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- Schuurmans JM, Rossell SL, van Tuijl A, Bakker BM, Hellingwerf KJ, Teixeira de Mattos MJ. (2008) Effect of h_{xk2} deletion and HAP4 overexpression on fermentative capacity in *Saccharomyces cerevisiae*. *FEMS Yeast Res.* 8(2):195-203.
- Punt PJ, Schuren FH, Lehmbeck J, Christensen T, Hjort C, van den Hondel CA. (2008) Characterization of the *Aspergillus niger* prtT, a unique regulator of extracellular protease encoding genes. *Fungal Genet Biol.* 2008 Dec;45(12):1591-9

Summary

- Several pH responding transcription factor deficient mutants were constructed and screened for protease activity and acid production
- Among others an interesting finding was that one mutant had an oxalic acid overproducing phenotype (OOP) and one mutant had indications of being protease deficient.
- The OOP mutant was further characterized in 2L scale bioreactors, and a 90 % (±25%) increase of the overall yield coefficient of oxalic acid on glucose was seen.