An automated method that enables high-throughput screening of knockout libraries with focus on industrial important metabolic cell properties

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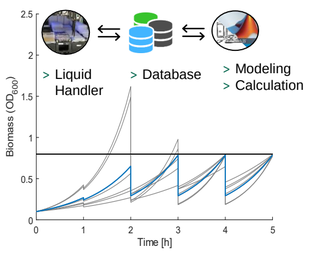
Introduction

An important area of process optimisation is the metabolic engineering of production strains. The modification of promoters, regulation by sRNA or targeted metabolic engineering are just a few of the diverse and creative solutions that bring about an increase in the organism's performance. Metabolic engineering includes the hypothesis that a genetically determined deactivation of unnecessary regulatory cascades or other synthesis pathways can bring about such a performance-enhancing effect. On the one hand, increasingly efficient genome editing strategies lead to large mutant libraries such as the Keio Collection, but on the other hand, there is a lack of methods to screen these large libraries for targeted purposes just as rapidly. This study focuses on filling this gap. Using the Quasi-Turbidostat (Hans et al., 2018), the plasmid-based reporter system pAG032 (Gawin et al., 2019) is transformed into a large selection of strains from the Keio Collection. This reporter system allows the observation of industrially important cell properties such as productivity, ribosomal capacity and metabolic stress responses. Subsequently, enzyme-based fed-batch screening is performed in the same 96-well plate scale.

Methods and Results

Quasi-Turbidostat

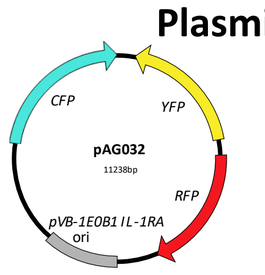


Synchronisation of biomass to ensure the early log phase state for cell competence

Cell competence and transformation

of 32 selected strains from the Keio Collection

Plasmid-based reporter system

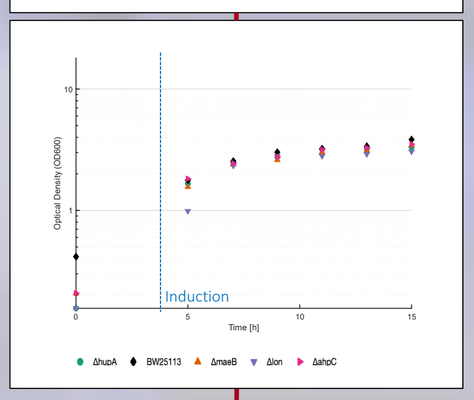


RFP ≙ Protein synthesis
YFP ≙ Ribosome synthesis
CFP ≙ Heat-shock like responses (HSR)

High-throughput screening

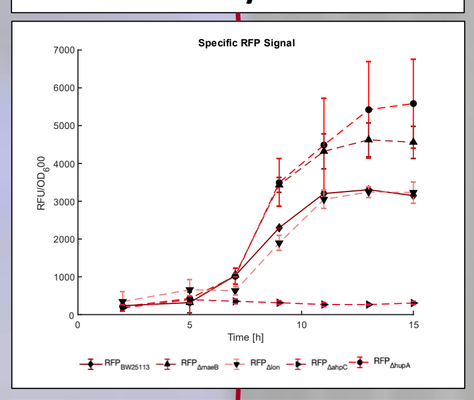
Enzyme-based fed-batch in 96 well plates with *E.coli* BW25113 as control strain. Monitoring of gowth, recombinant protein synthesis, ribosome synthesis and stress response (heat shock response) via *at line* fluorescence measurements.

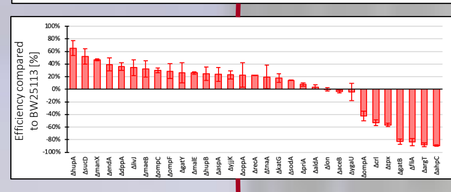
Growth



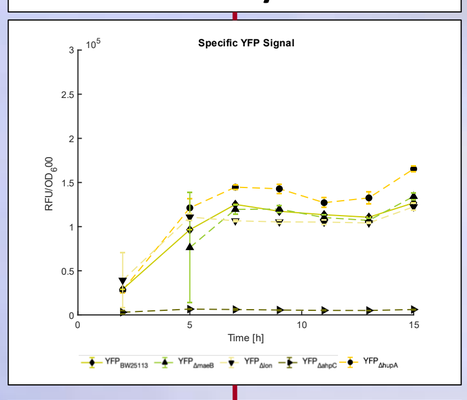
No significant differences in growth
(In this study)

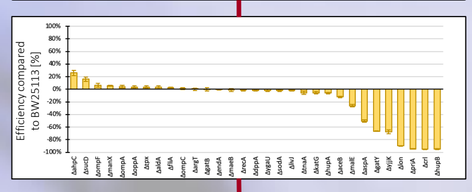
Protein synthesis



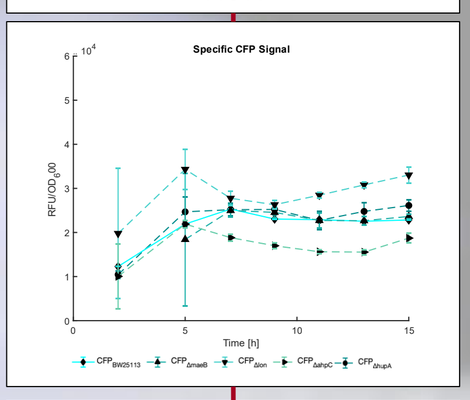


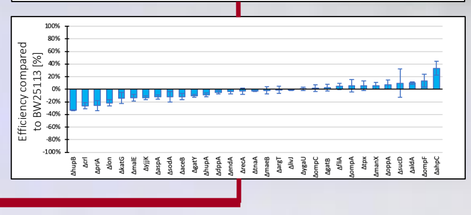
Ribosome synthesis





HSR





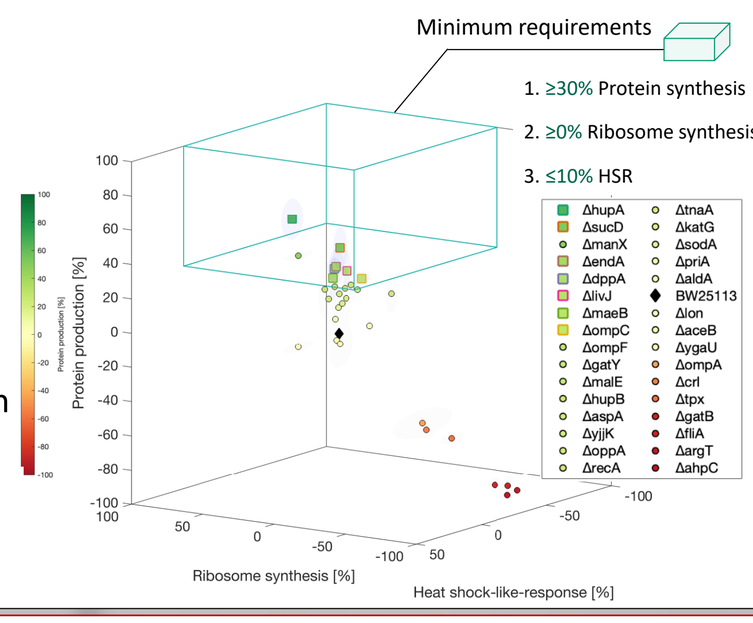
Strain discrimination

Many gene deletions have significant effects on one or more cell parameters. These will now be considered in their entirety in order to be able to evaluate the effect on the set focus.

Strain	Efficiency (compared to BW25113) RFP (Productivity) Value	RFP STD	YFP (Ribosome Synthesis) Value	YFP STD	CFP (HSR) Value	CFP STD
ΔhupA	65,38%	11,98%	26,00%	4,30%	7,50%	6,91%
ΔmanX	46,50%	1,54%	16,12%	3,55%	13,55%	10,18%
ΔdppA	36,18%	6,38%	6,55%	2,91%	-3,65%	3,82%
ΔgatY	26,14%	16,24%	5,72%	0,50%	4,72%	4,81%
ΔpriA	7,57%	3,00%	4,18%	2,12%	-1,82%	5,76%
ΔmaeB	32,08%	12,79%	3,64%	2,15%	0,69%	2,76%
ΔhupB	24,97%	10,63%	3,50%	1,92%	-13,39%	5,06%
ΔsodA	14,12%	0,29%	3,14%	2,14%	-3,02%	4,87%
ΔlivJ	34,29%	12,31%	2,35%	0,86%	-8,86%	2,87%
ΔoppA	22,77%	19,75%	0,89%	1,57%	-1,14%	5,69%
ΔaceB	-3,55%	2,01%	0,09%	1,98%	1,68%	5,17%
ΔendA	39,51%	10,31%	-0,09%	2,68%	2,39%	5,41%
ΔtnaA	19,63%	18,92%	-0,80%	0,02%	-4,83%	2,73%
ΔyjjK	22,78%	6,30%	-1,30%	2,19%	-13,17%	3,10%
ΔkatG	17,51%	6,80%	-1,32%	1,35%	-0,98%	0,93%
ΔmalE	25,95%	1,98%	-1,61%	1,01%	-2,85%	0,60%
ΔrecA	22,65%	0,03%	-1,67%	2,01%	9,83%	2,24%
Δlon	-0,08%	2,71%	-1,88%	1,62%	33,34%	11,32%
ΔompF	28,82%	12,10%	-1,99%	0,52%	5,51%	7,46%
ΔompC	30,00%	3,79%	-5,50%	2,85%	-11,74%	4,91%
ΔygaU	-4,22%	13,54%	-5,71%	1,87%	5,40%	9,91%
ΔsucD	51,94%	12,37%	-6,06%	0,96%	5,88%	5,23%
ΔaldA	3,64%	3,64%	-12,24%	1,05%	-10,35%	2,47%
ΔaspA	23,59%	11,19%	-26,13%	2,12%	-11,95%	3,46%
Δcrl	-52,97%	4,75%	-50,55%	1,89%	-11,90%	8,51%
Δtpx	-56,56%	2,86%	-66,38%	0,63%	-13,95%	8,59%
ΔompA	-42,23%	7,60%	-67,52%	3,19%	9,66%	22,62%
ΔgatB	-83,05%	4,14%	-89,70%	0,09%	-21,73%	4,90%
ΔahpC	-89,26%	1,01%	-94,76%	0,41%	-25,92%	7,88%
ΔfliA	-83,87%	5,89%	-94,96%	0,41%	-26,05%	4,74%
ΔargT	-88,00%	3,35%	-95,19%	0,70%	-33,01%	0,71%

Data

Merging
Filtration
Visualisation



- 32 selected strains from the Keio Collection were simultaneously transformed with pAG032
- 7 mutants (□) reached the minimum requirements and show interesting gene deletions for a multi knockout strain
- 65% increased protein synthesis by deletion of one gene (*hupA*)
- 26% increased ribosome synthesis by deletion of one gene (*hupA*)
- No significant differences in growth

Conclusions and Outlook

i. Automated high throughput transformation strategies and the use of the pAG032 reporter system allow rapid screening of mutant libraries and other microorganisms with industrial relevant metabolic cell properties.
ii. The efficiency of a production strain cannot be defined by growth alone.
iii. The elimination of genes or unneeded regulatory cascades has a significant effect on recombinant protein production.
iv. This method combined with data science could enable targeted gene function analysis.
v. A verification and validation of the results in stress-like conditions of a production scale is necessary.

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