Metabolic engineering of *E. coli* for the sustainable production of short-chain esters

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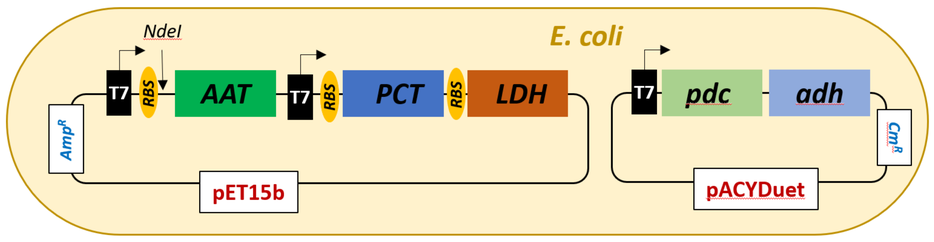
BACKGROUND

Aim: Engineering *E. coli* for ethyl lactate/acetate production

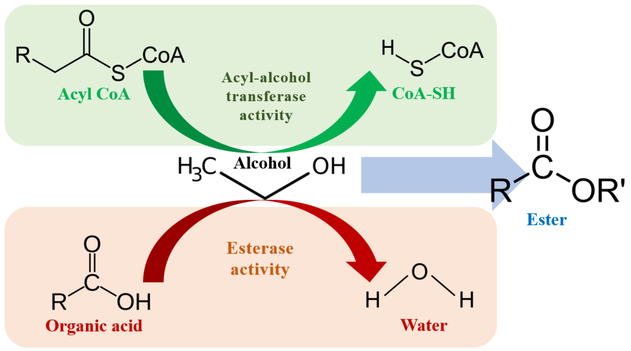
Steps:

1.Bioprospecting of ester producing enzyme genes
2.Construction of recombinant *E. coli* cell factories
3.In vitro analysis of esterase activity
4.High cell density fermentation and optimization

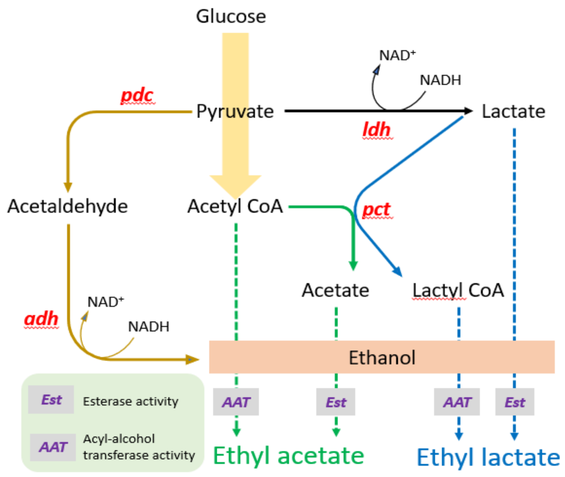


Activities under consideration

- Acyl-alcohol transferase activity
- Esterase activity



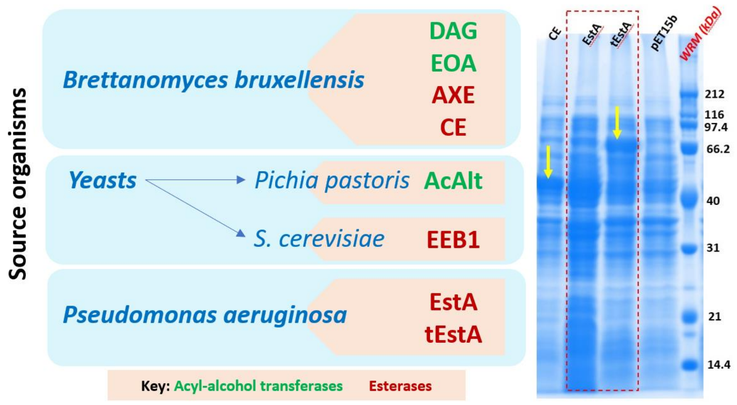
Engineered metabolic pathway



BIOPROSPECTING OF GENES

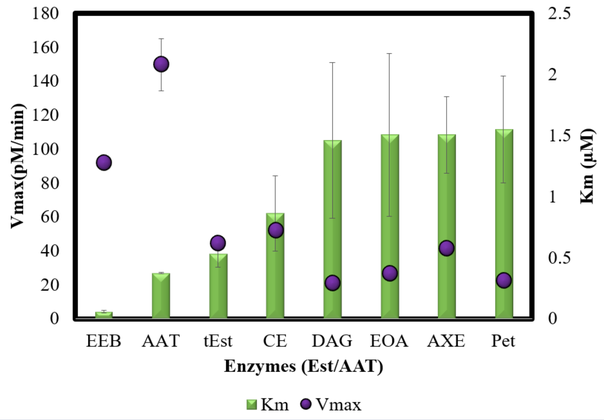
Genes under consideration:

Un-annotated *Brettanomyces* genes were curated based on sequence homology. All genes were codon optimized.



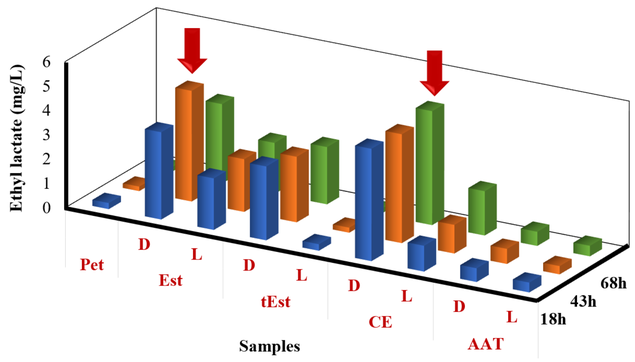
Esterase assay:

To determine ester hydrolysis activity of the enzymes under consideration.



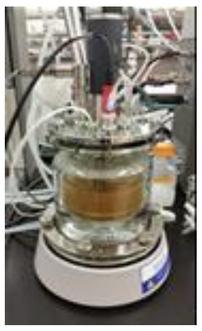
PRELIMINARY SCREENING

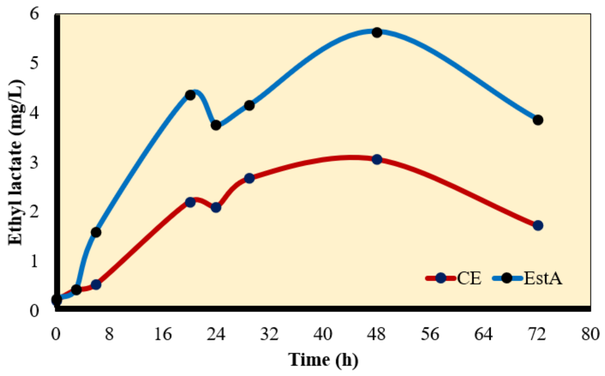
4 efficient candidates were selected: EstA, tEstA, CE and AAT.
Cell density: 10.0 (OD_{600})

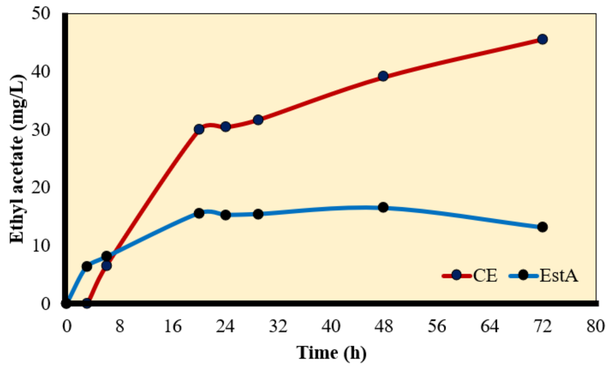


REACTOR STUDIES

Cell density: 3.0 (OD_{600})
Bioreactor (batch) studies showed higher ethyl lactate titers with EstA possessing strains while higher ethyl acetate titres were obtained with those with CE..







CONCLUSION

Conclusion:

- Successful demonstration of ethyl lactate and ethyl acetate production from *E. coli* using esterases.
- *In vivo* or *in vitro* routes can be efficiently explored for ester biosynthesis.

Inference:

- Esterases can be exploited for esterification.
- Small-, medium-, long- chain esters can be targeted through biocatalysis.

FUTURE ASPECTS

- *E. coli* has its metabolic limitations on efficient conversion of metabolic substrates to esters. Hence, other microbial systems can be explored for efficient production of these esters.
- *E. coli* platform can be further investigated and engineered for production of higher alcohol partners for ester biosynthesis.

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