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## INTRODUCTION

### Abstract

Esters and their precursors are economically viable hydrocarbons, especially known for their biodegradable properties. With the aim of synthesizing these green chemicals through a greener process we chose a biocatalysis route. Spontaneity of esterification reactions was analysed through a web-based eQuilibrator tool by calculating their Gibbs free energy change. Simultaneous investigation was performed to mine for various esterase and acyl-alcohol transferase enzymes. Protein database search, sequence (*blastp*) and structure (TM-align) alignments yielded three functionally related but structurally distinct enzymes, namely ATF1, ATF2 and LgATF1. Further *in silico* and *in vitro* laboratory studies can confirm their functionality in ester biosynthesis.

### Motivation

- Increasing the production of sustainable solvents such as ethyl lactate.
- The economic possibilities of its production as a green solvent is very consequent.
- Using agricultural products as raw materials
- Exploring more such genes that can improve its yield.
- Exploring new esterase or acyl alcohol transferase genes
- Comparing amino acid sequences that can improve the production of esters.

Goal: Finding efficient way to produce ethyl lactate

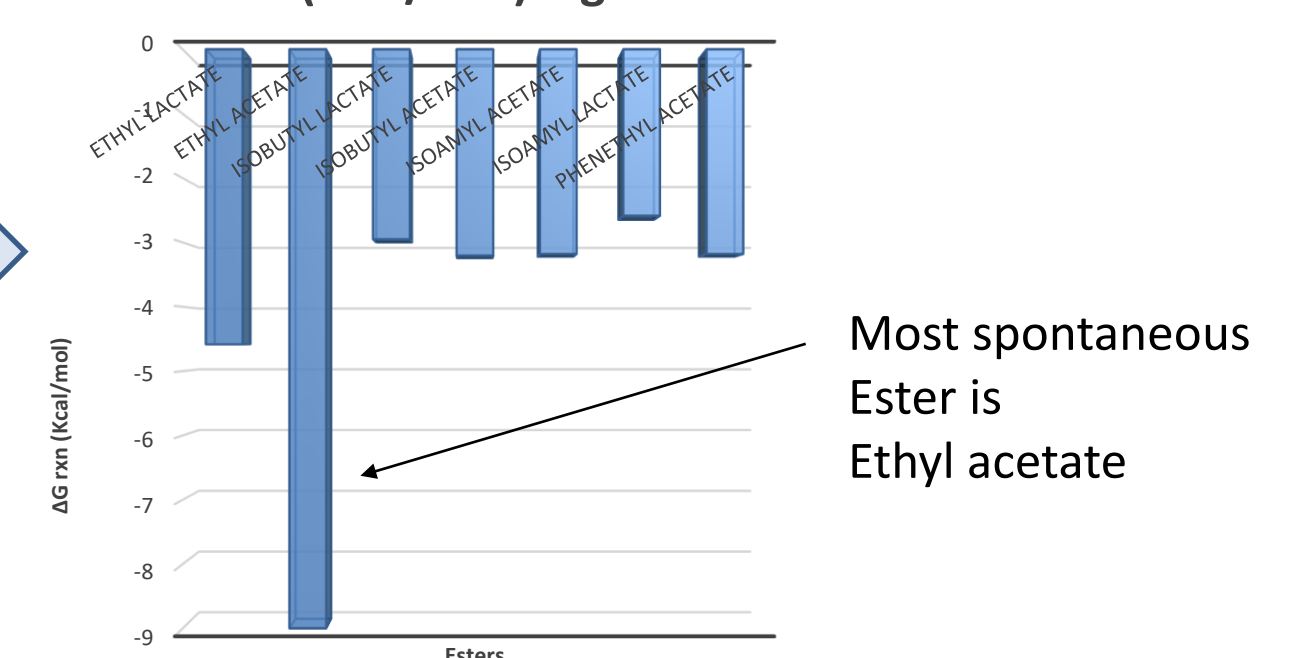
## RESULTS AND DISCUSSION

### Section 1: $\Delta G$ values Esterification reaction $A+B \rightarrow C+D$

A: alcohol C: ester  
B: organic acid D: water

Esters:	$\Delta G$ rxn (Kcal/mol)
ethyl lactate	-4,584130019
ethyl acetate	-8,996175908
Isobutyl lactate	-2,949330784
Isobutyl acetate	-3,202676864
Isoamyl acetate	-3,178776291
Isoamyl lactate	-2,586042065
Phenethyl acetate	-3,178776291

$\Delta G$  rxn (Kcal/mol) against Esters



### Section 2: Gene mining for biochemical esterification

#### 2a. Genes Under Investigation

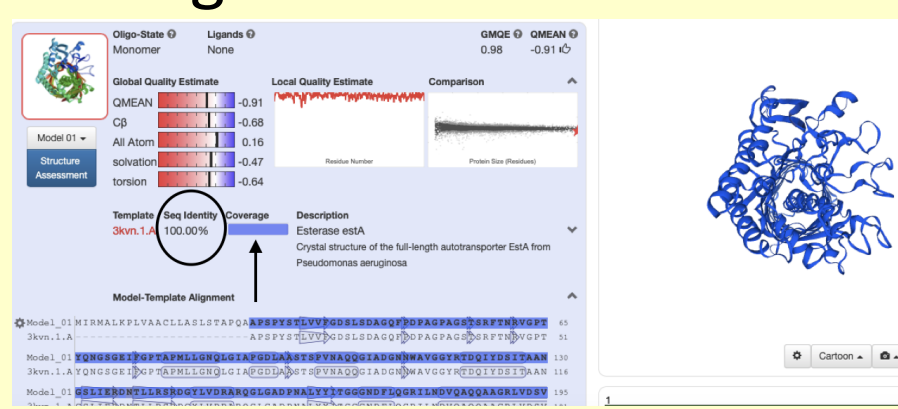
- EsteraseA (EstA)
  - Biotin (BioHC)
- From *Pseudomonas aeruginosa*

- Alcohol O-acetyltransferase 1 (ATF1)
  - (ATF2)
  - Medium-chain fatty acid ethyl ester synthase/esterase 1 (EEB1)
- From *Saccharomyces cerevisiae*

- Lg-ATF1
- From *Pseudomonas pastorianus*

#### 2b. Esterase screening based on the known esterase EstA

##### Structure analysis of EstA using SWISS MODEL



##### Comparison of amino acid sequences based on their EC number

##### EstA and BioHc

Sequence ID	Query	Length	Number of Matches
Range 1	394 to 413	Graphics	1
Score	15.8 bits(29)	4.7	Compositional matrix adjust.
Query	353 ELRSQKADENRQVQVRG	373	→ EstA
Sbjct	394 ELKRAQ-QVYVHVRVYG	413	→ BioHc

#### 2c. ATF1 and ATF2

Sequences producing significant alignments	Description	Max Score	Total Query Score	Per. Ident.	Accession
<input checked="" type="checkbox"/>	unannotated protein product	308	305	99%	tr L21145 Query_11100

#### ATF1 and Lg-ATF1

Max Score	Total Query Score	Per. Ident.	Accession
897	897	100%	0.00 (90.81%) Query_901

#### Multiple sequence alignment using Clustal Omega

Percent Identity Matrix - created by Clustal2.1

	1:	sp P53296 ATF2_YEAST	100.00	36.10	35.65
ATF1	2:	sp P40353 ATF1_YEAST	36.10	100.00	80.34
Lg-ATF1	3:	tr Q6XBS9 Q6XBS9_SACPS	35.65	80.34	100.00

There are some level of sequence similarity between ATF1 and Lg-ATF1 in *Saccharomyces cerevisiae*.

## CONCLUSION AND ACKNOWLEDGEMENT

Successful bioinformatics research in *Saccharomyces cerevisiae*. Future project: explore these genes in *Wickerhamomyces*.

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