

# Designing a consolidated microbial process for production of bioethanol from *Ulva* biomass

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## INTRODUCTION:

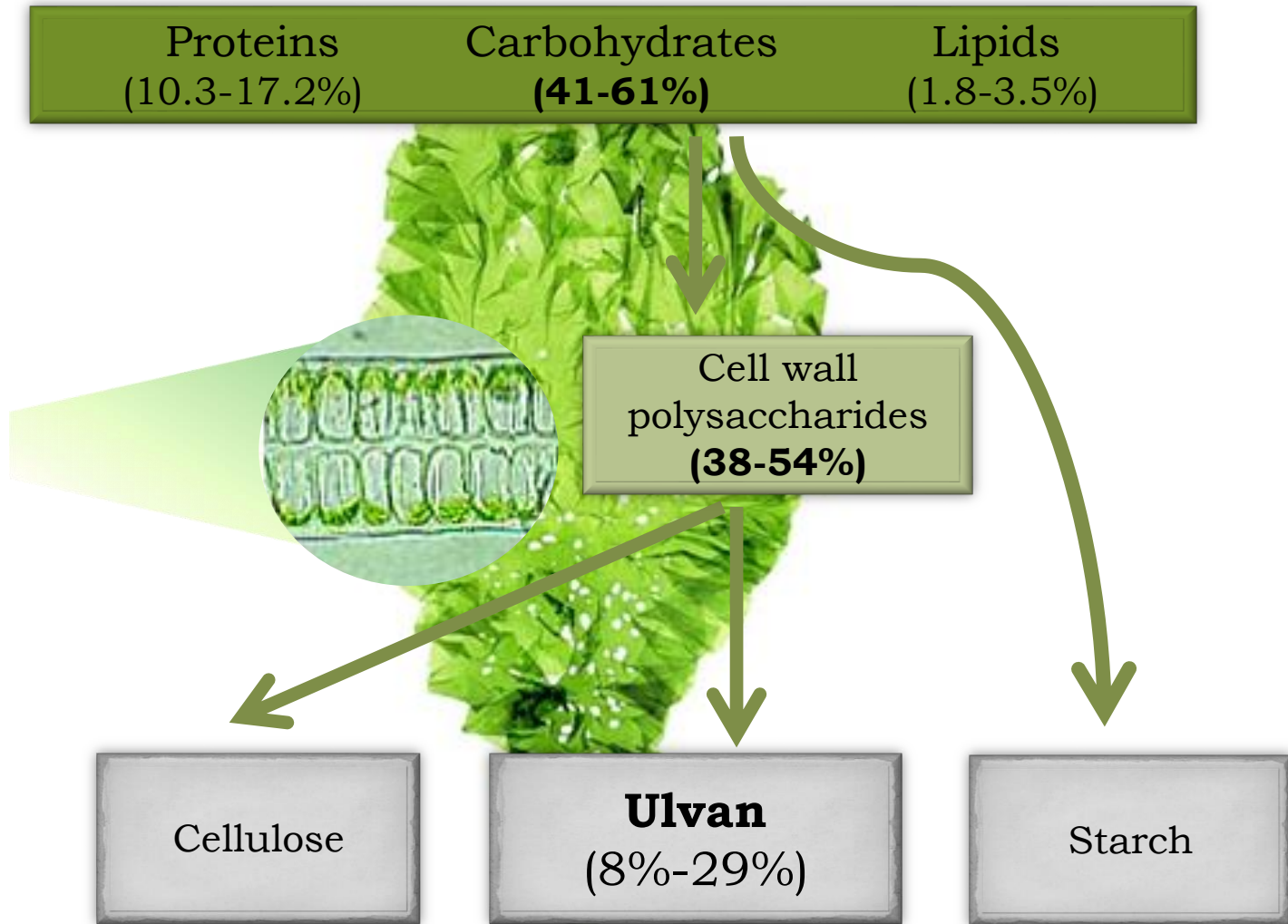
# Bioethanol as alternative energy

- Of the major sustainable energy options, biofuel is most promising
  - ❖ **Bioethanol** – enzymatically produced fuel from fermentable sugars
- Versatile biofuel feedstock: **ALGAE**
  - ❖ Green algae is an underexploited resource

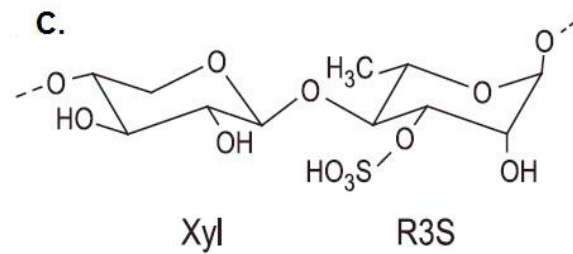
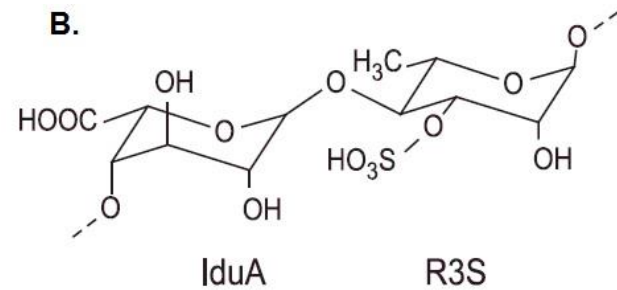
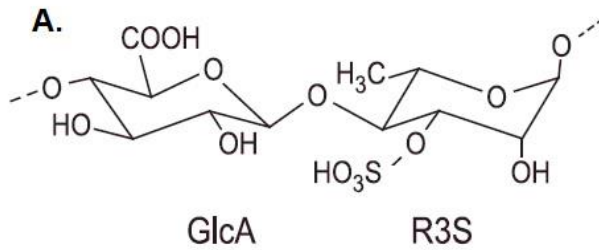
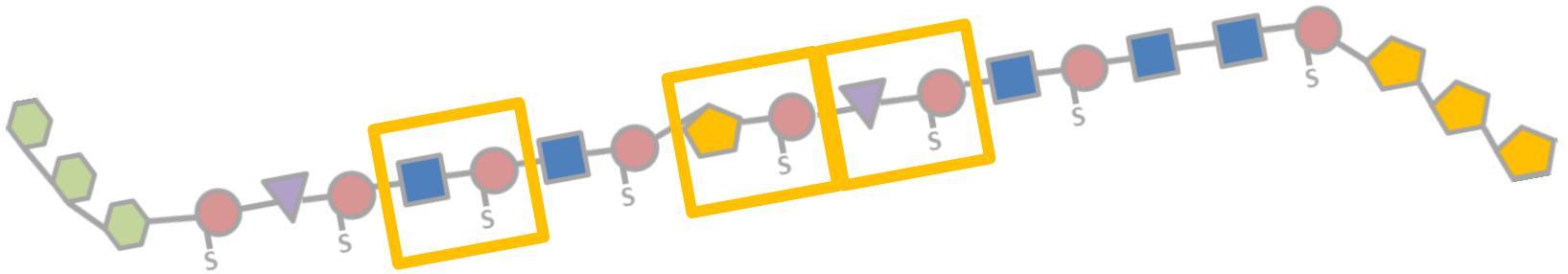







# INTRODUCTION :

## *Ulva* sp. as source of bioethanol



# INTRODUCTION : Ulvan structure



-  Glucose (Glu)
-  Xylose (Xly)
-  Glucuronic acid (GluA)
-  Rhamnose 3-S (Rha3S)
-  Iduronic acid (IduA)

(Robic *et al.* 2009)

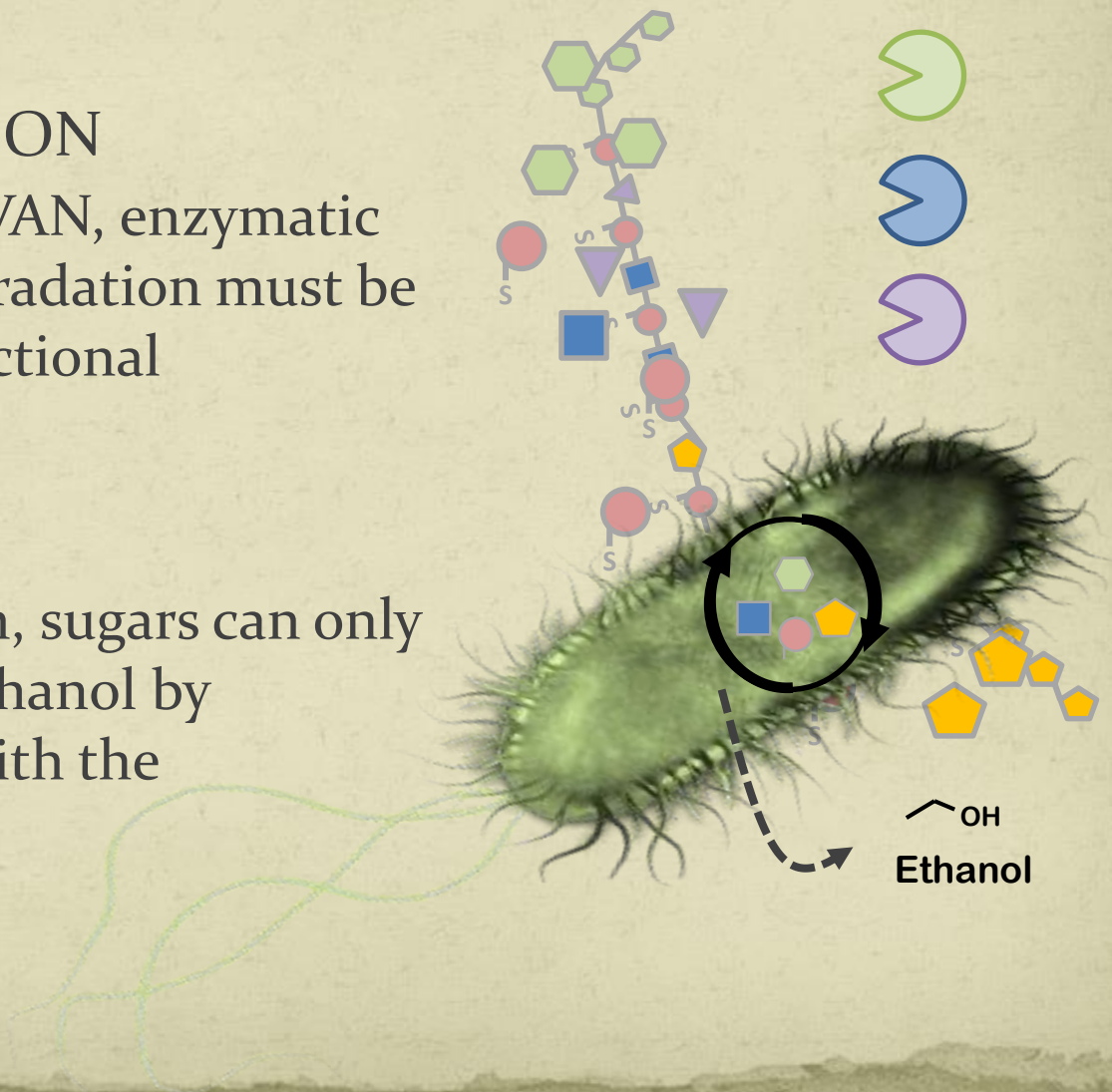
# INTRODUCTION: Biomass to bioethanol

## 1. SACCHARIFICATION

- ❖ To fully utilize ULVAN, enzymatic machinery for degradation must be identified and functional

## 2. FERMENTATION

- ❖ Once broken down, sugars can only be fermented to ethanol by microorganisms with the appropriate tools

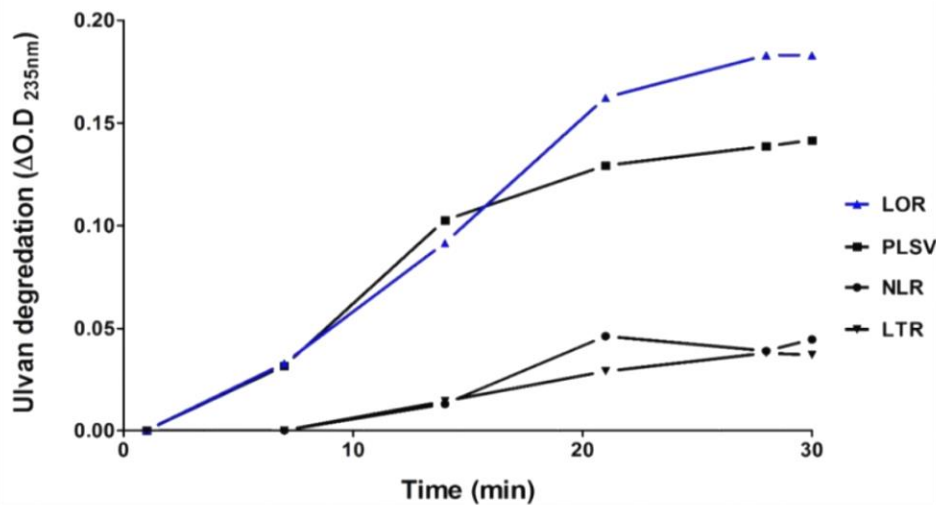


Part I

Optimize enzymatic machinery for  
ulvan saccharification

# CHAPTER 1: Native ulvan-degrading bacteria

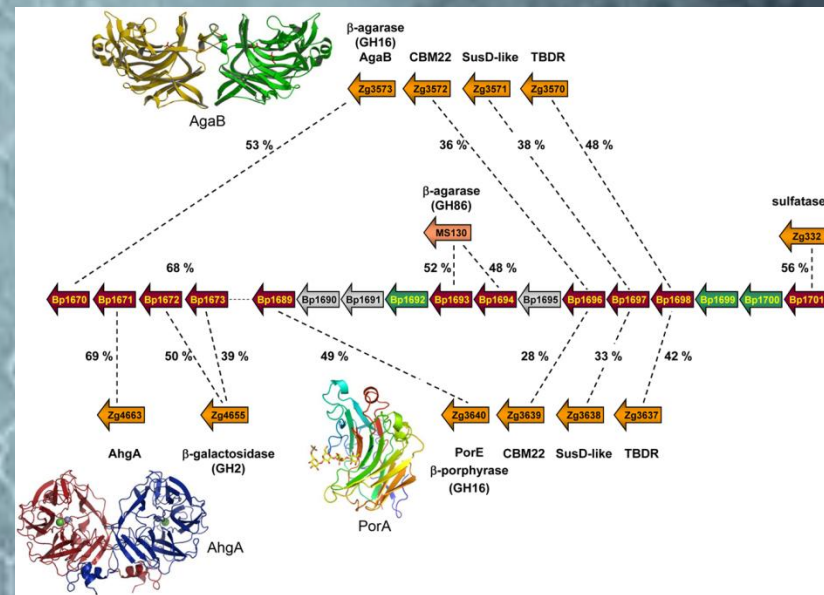
- Four bacteria were isolated from *Aplysia* sp. and tested for ulvan/oligo-degrading capability
  - ❖ In collaboration with Dr. William Helbert from CERMAV-CNRS, France



- Alteromonas sp.(LOR)
- Pseudoalteromonas sp.(PLSV)
- Alteromonas sp.(LTR)
- Flavobacterium sp.(NLR)

# CHAPTER 1: Polysaccharide utilization locus (PUL)

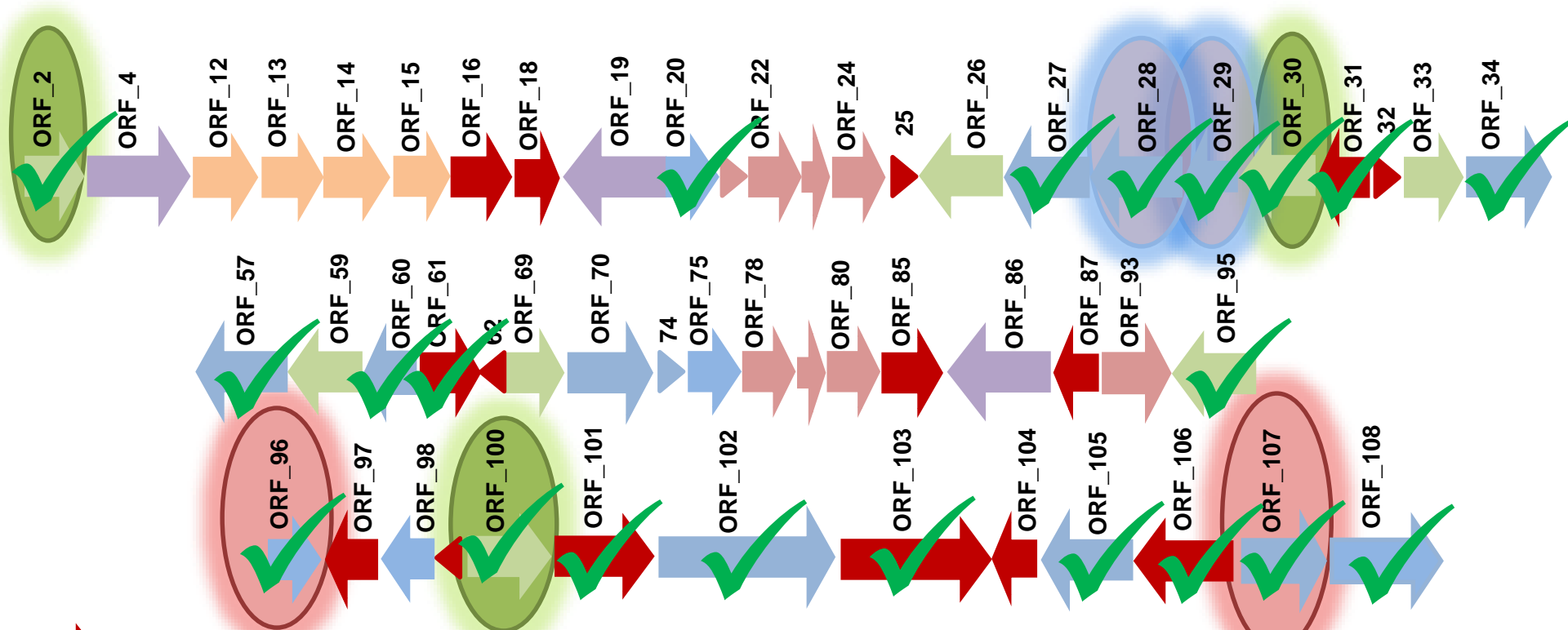
- Genomes of the native marine bacteria were sequenced and annotated, revealing ulvan PULs
- A PUL is a set of physically linked genes that coordinates the breakdown of a specific polysaccharide
  - ❖ Includes genes for sensing and binding the polysaccharide, to cleavage and transport of oligosaccharides









Thomas *et al.* 2011, *Front. Microbiol.*



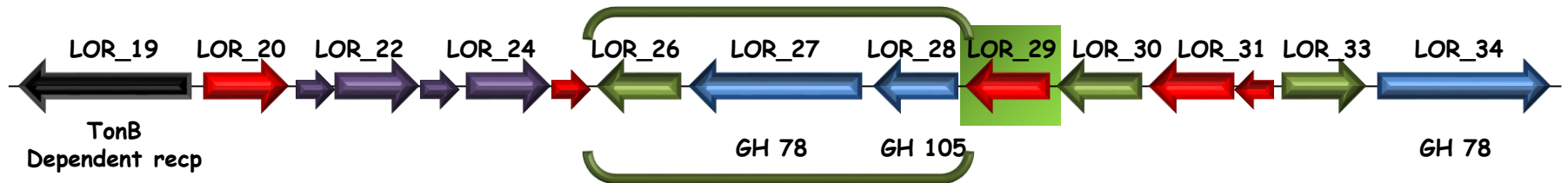
# PULs found in LOR ulvan cluster



-  Hypothetical protein
-  Polysaccharide sensing, binding and transport proteins
-  Proteins involved in polysaccharide degradation (GH and PL)
-  Xylose metabolism
-  Rhamnose metabolism
-  Sulfatase

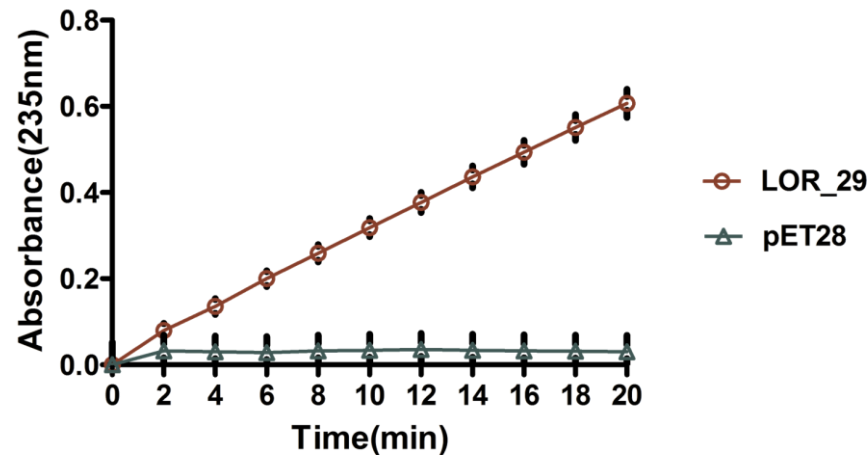
# Detection of novel ulvan lyase

## The first enzyme required for ulvan degradation



hypothetically involved in oligo-ulvans degradation

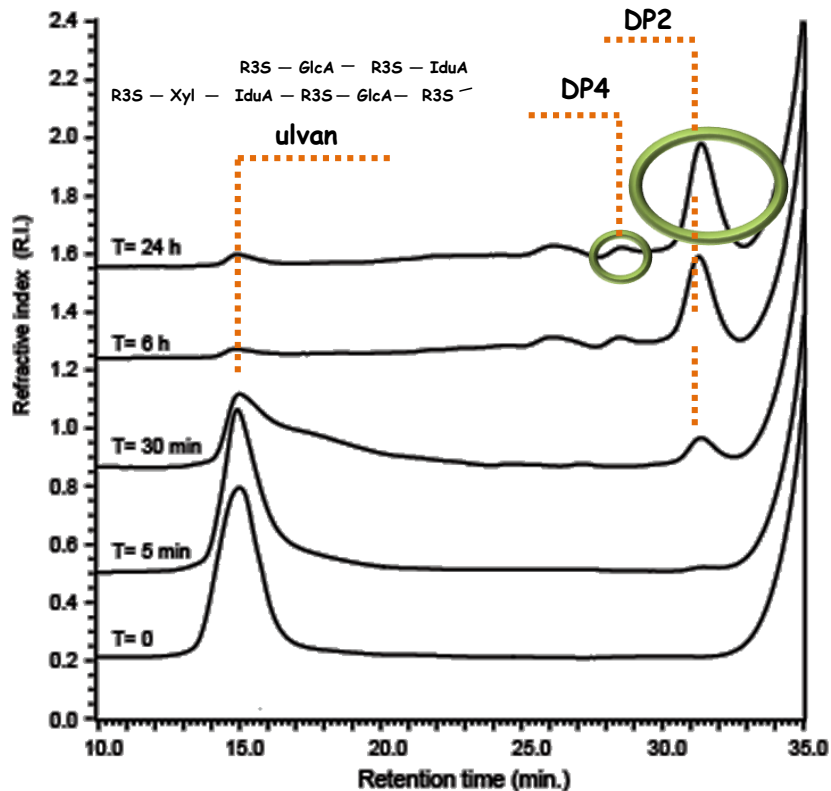
incubated with ulvan



# Degradation kinetics of LOR\_29

Ulvan + purified LOR\_29 protein

analytical size-exclusion chromatography



Two main products:

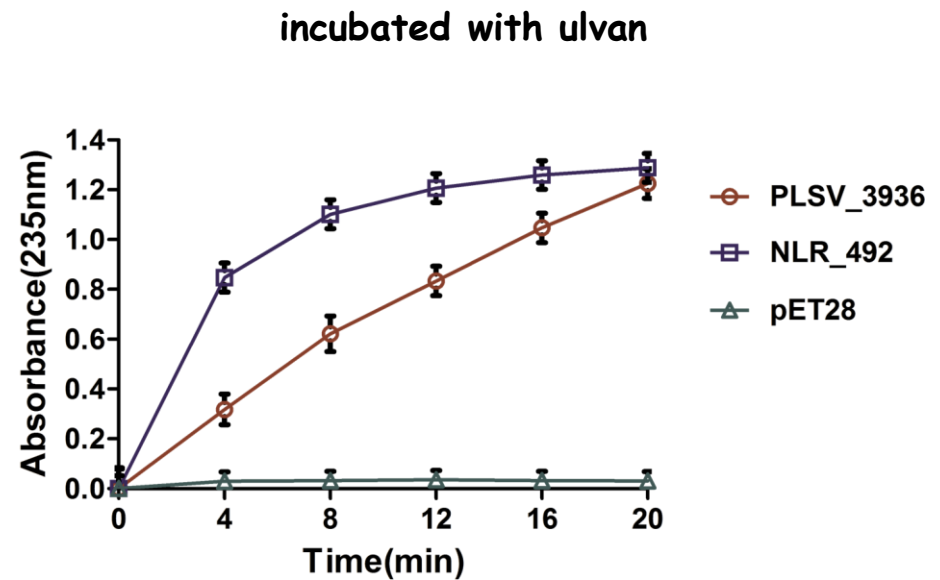
DP2 - disaccharide  $\Delta$ GlcA-R3S

DP4 - tetrasaccharide  $\Delta$ GlcA-R3S-Xyl-R3S

Verified by  $^1\text{H-NMR}$  spectra analysis

# LOR\_29 homologs

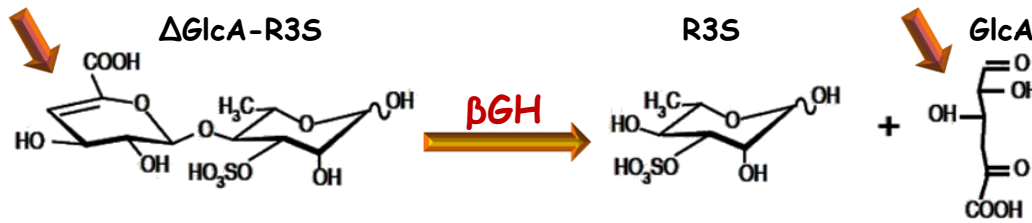
Strain	Pair similarity	coverage
<i>Pseudoaltermonas</i> sp. PLSV	76%	97%
<i>Altermonas</i> sp. LTR	100%	100%
<i>Nonlabens ulvanivorans</i> (NLR)	53%	99%
<i>Paraglaciecola agarilytica</i>	76%	97%
<i>Paraglaciecola chathamensis</i>	75%	97%
<i>Catenovulum agarivorans</i>	61%	96%
<i>Pseudoalteromonas haloplanktis</i>	57%	98%



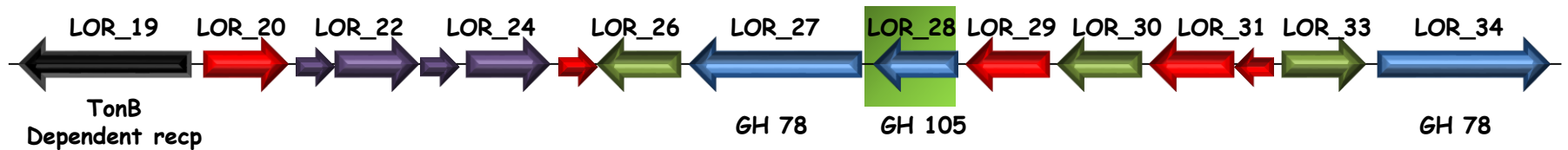
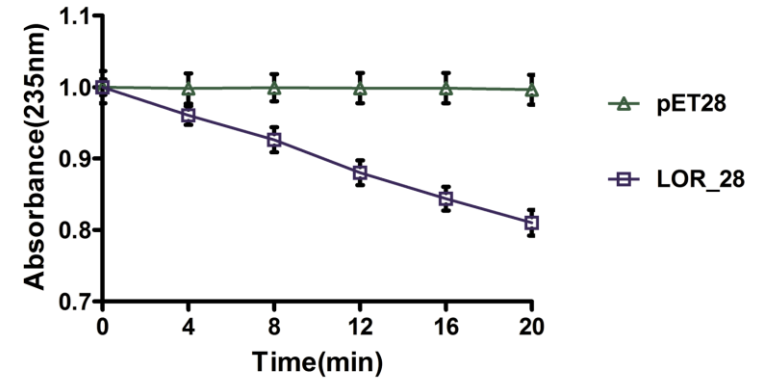
# Second enzyme in ulvan saccharification process

## $\beta$ - glucuronyl hydrolase ( $\beta$ GH)

- ❖ Relates to GH105 or to GH88 families
- ❖ Digests products of ulvan lyases (oligo-ulvan)
- ❖ Degradation decreases absorption (235<sub>nm</sub>)

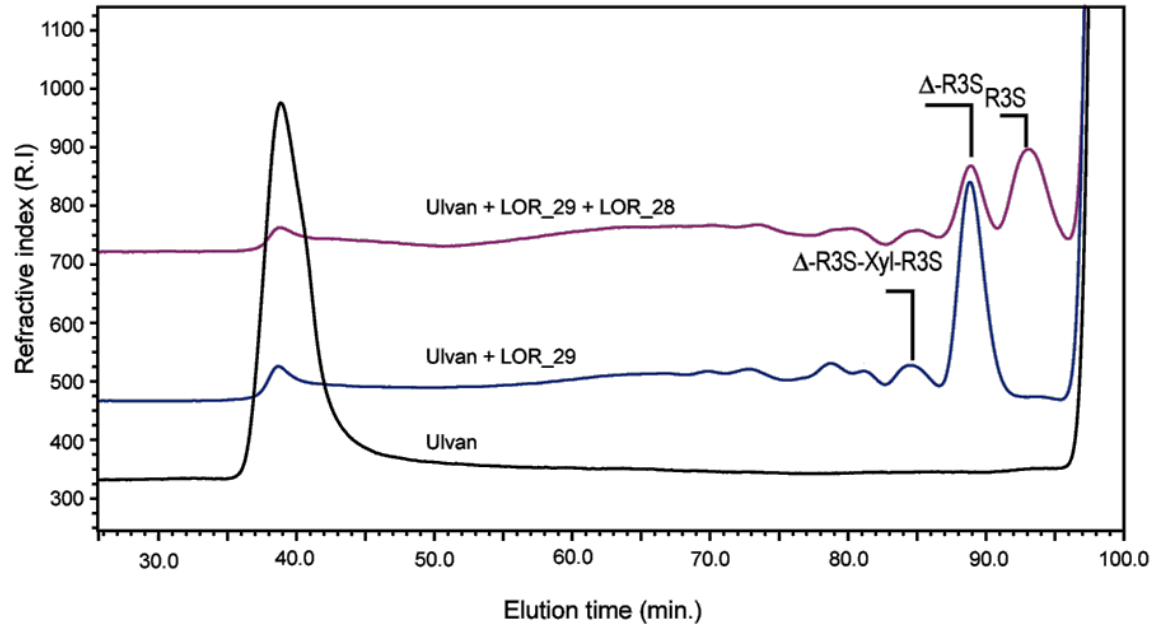


incubated with ulvan-modified by LOR\_29



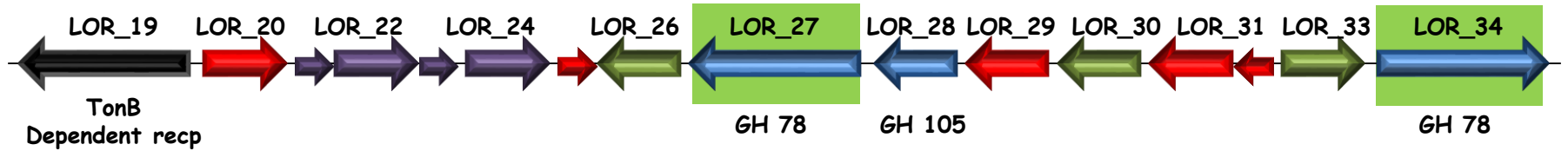
# LOR\_28 characterization

ulvan-modified by LOR\_29 + purified LOR\_28

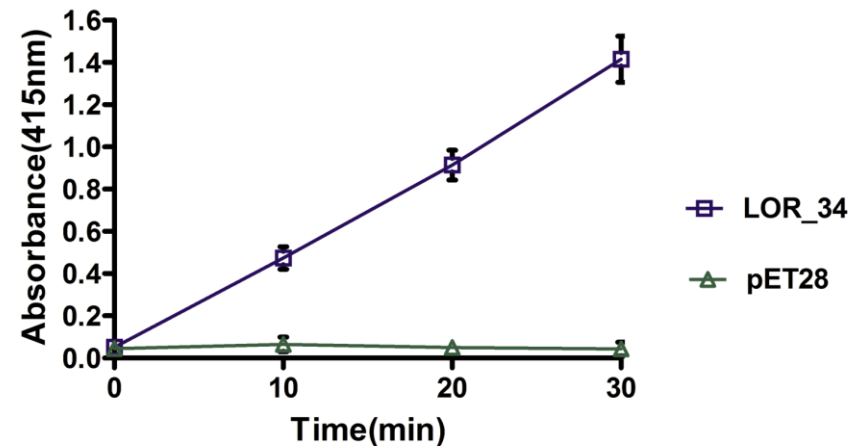


- ❖ LOR\_28 is able to remove unsaturated glucuronyl residue ( $\Delta$ -GlcA)
- ❖ LOR\_28 is active on LOR\_29 products

# Identification of rhamnosidases

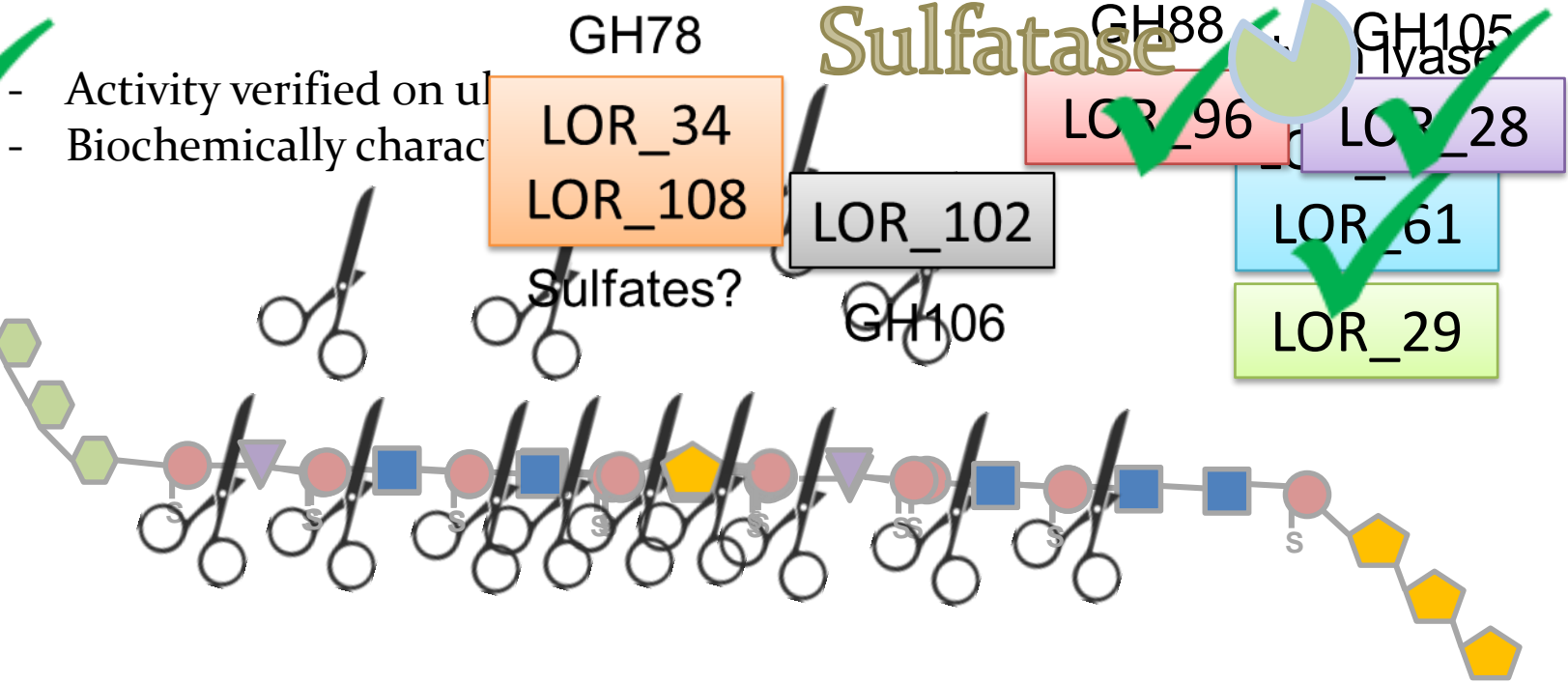


- ❖ GH 78 - family of proteins with rhamnosidase activity
- ❖ LOR\_27 - not enough soluble protein
- ❖ LOR\_34 - able to release 4-nitrophenol leaving group



# Proposed ULVAN saccharification

The MAIN players:



- Activity verified on ulvan
- Biochemically characterized

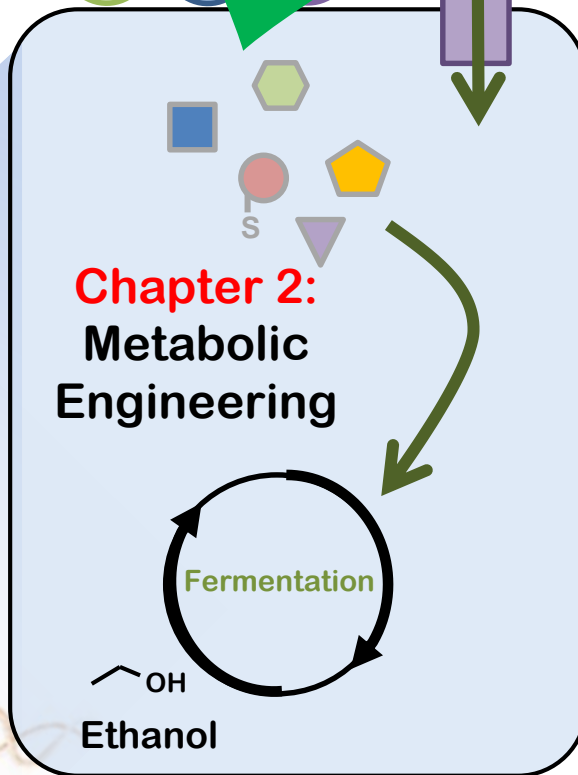
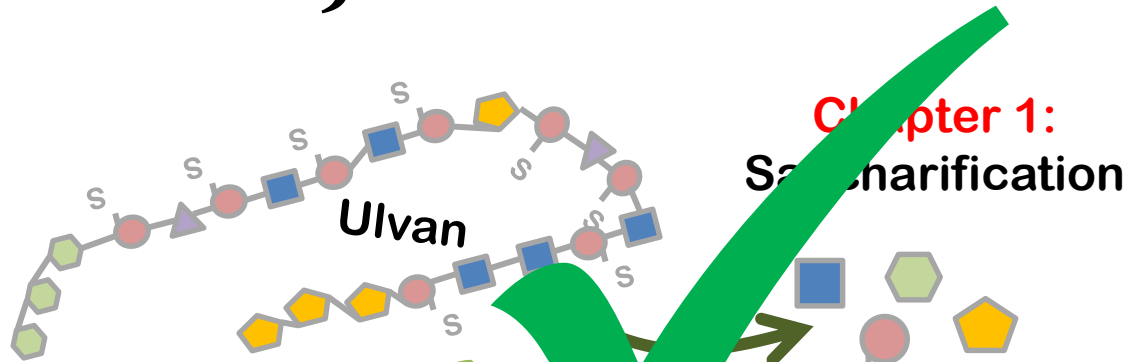
Leaving groups



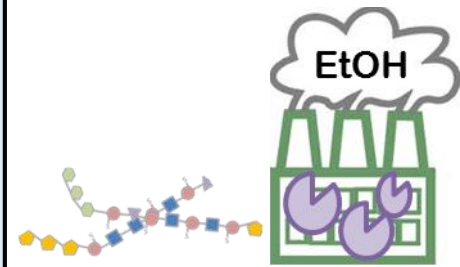
Iduronic acid (IduA)	Xylose (Xly)	Rhamnose 3-S (Rha3S)
Glucuronic acid (GluA)	Glucose (Glu)	



# PROJECT OVERVIEW



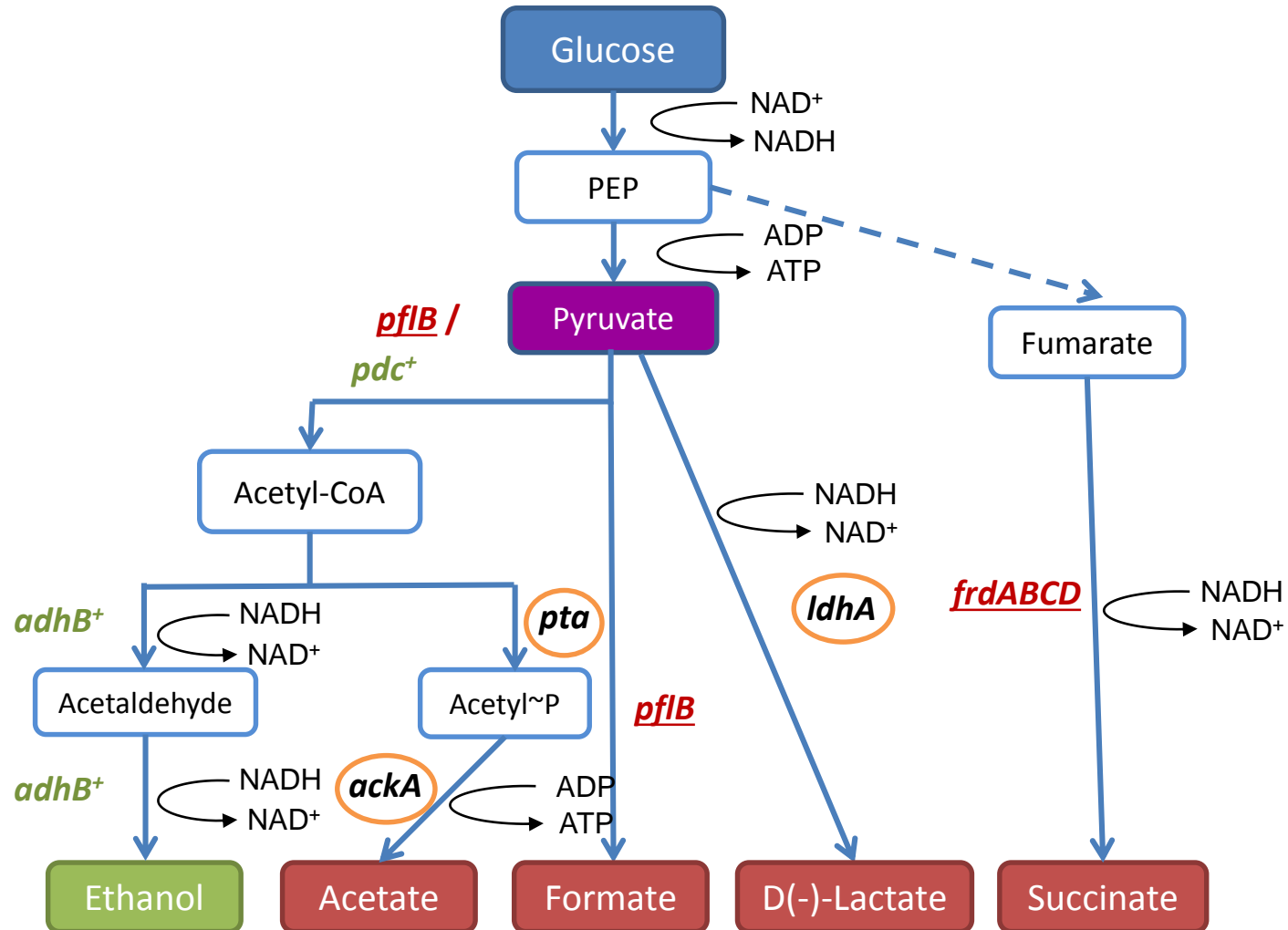
Chapter 3: Consolidated Bioprocess (CBP)



*E. coli*

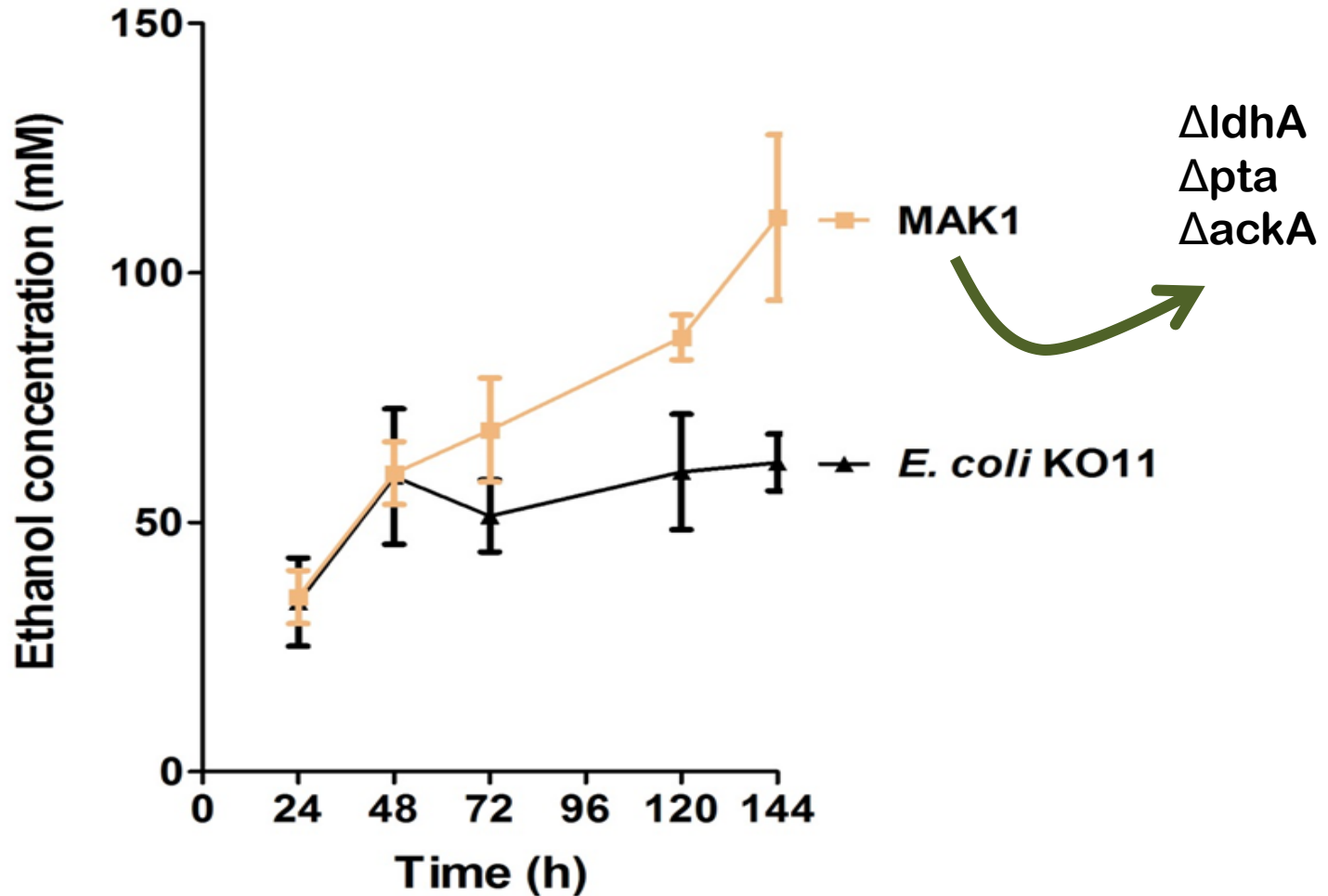
CHAPTER 2:  
Engineer metabolic pathways for ethanol fermentation of  
ulvan building blocks

# CHAPTER 2: Fermentation in *E. coli*



# Metabolic engineering

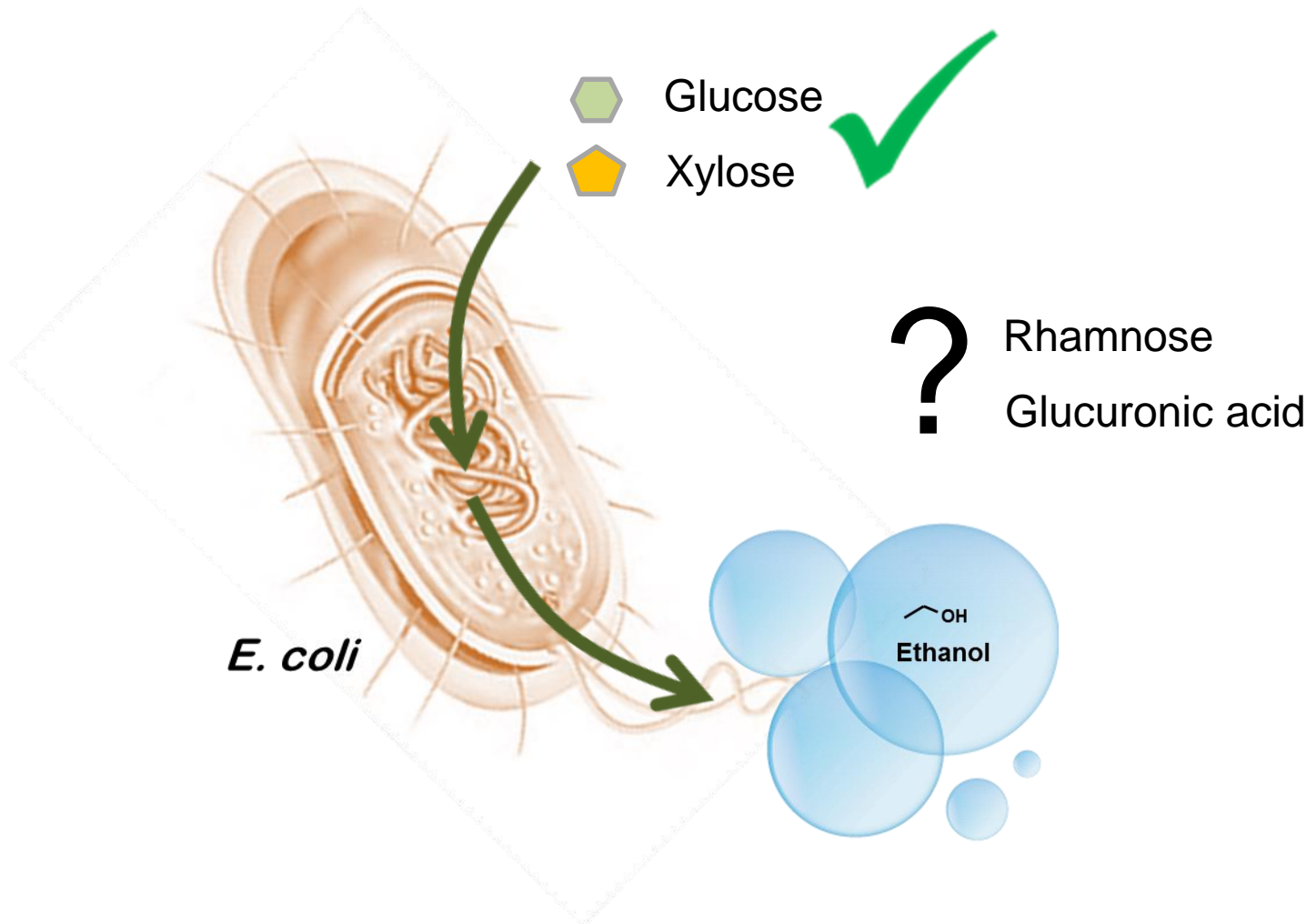
## Optimization of ethanol fermentation



(Mariana Klyman, unpublished)

CHAPTER 2:

# Optimization of ethanol fermentation



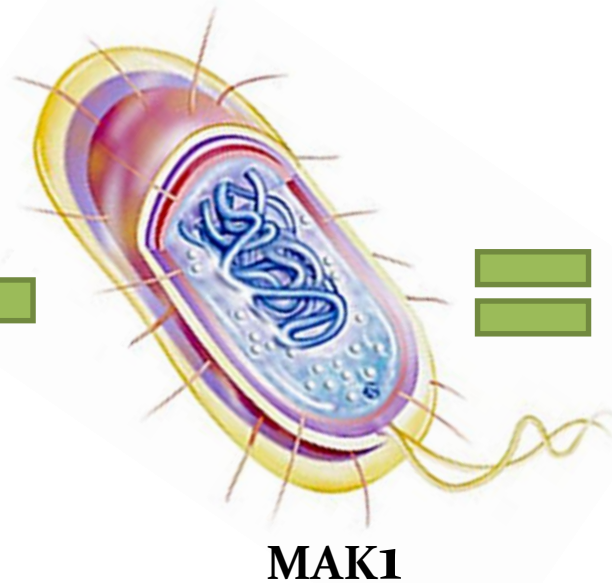
# CHAPTER 2: Coupled fermentation



*rhaA, rhaB,  
rhaD, aldA, ldhL*



*kduL, kduD,  
kdgK, eda*



**Coupled  
Fermentation**

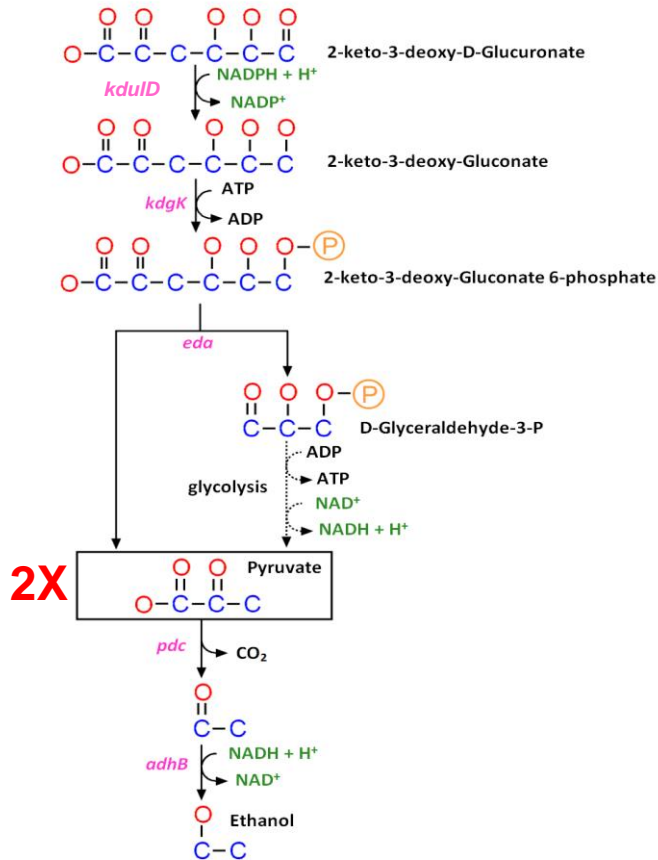
# Metabolic engineering of fermentation pathway

- Glucuronic acid:

*kduID*

*kdgK*

*eda*



- Rhamnose:

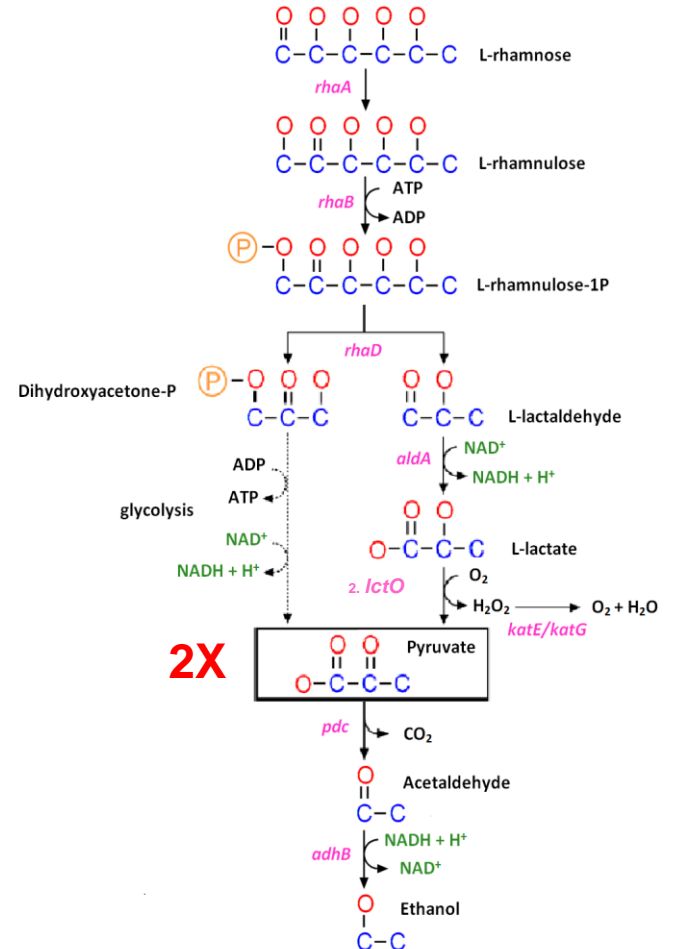
*lctO*

*rhaA*

*rhaB*

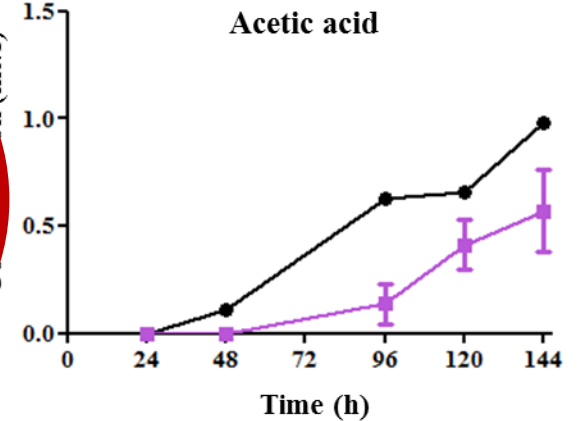
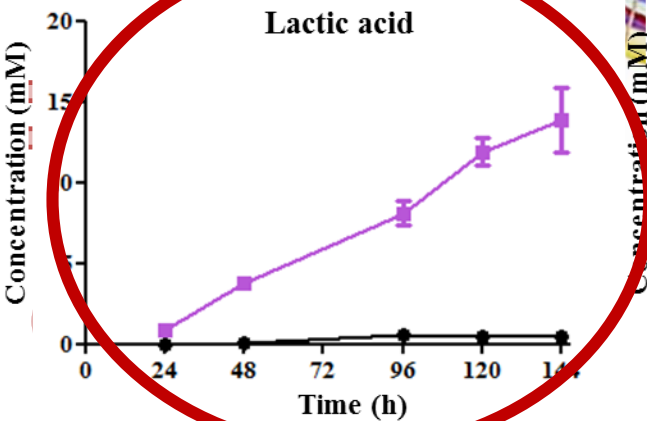
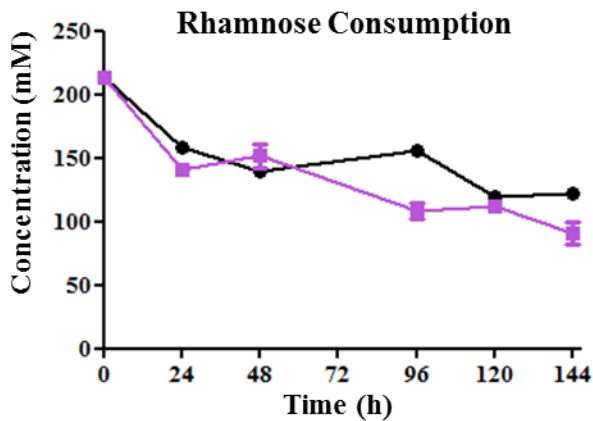
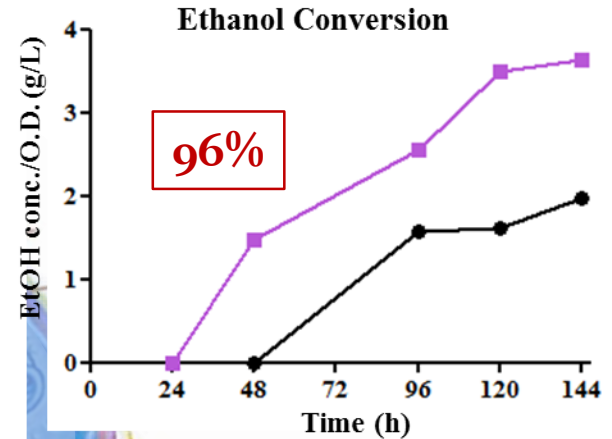
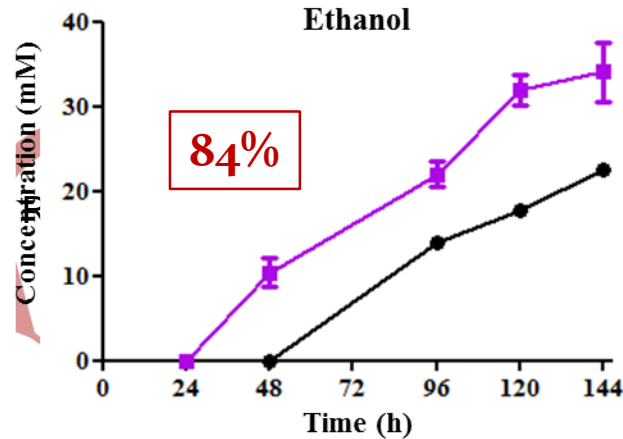
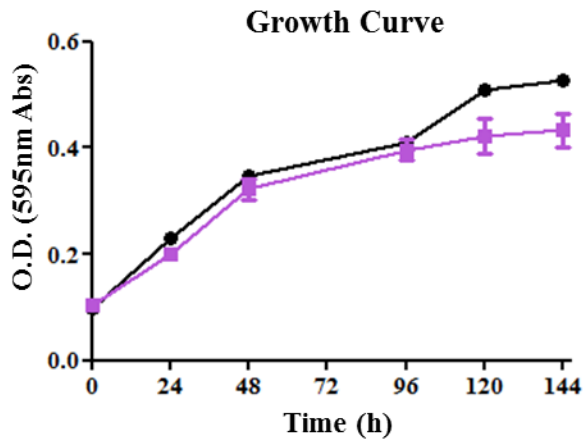
*rhaD*

*aldA*



# Metabolic engineering

## Rhamnose fermentation



● MAK1/vector  
■ MAK1/vector+Rham1

fermentation



# Summary

- Identified and expressed most of the enzymes required for saccharification of ulvan
- Improved rhamnose fermentation

# Acknowledgments

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