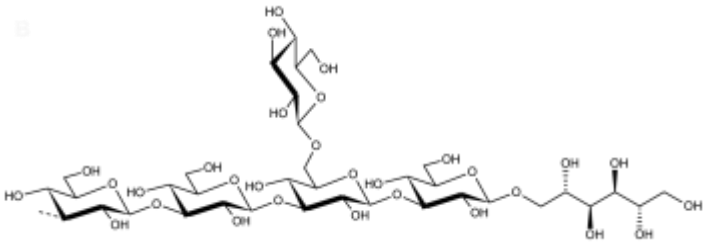
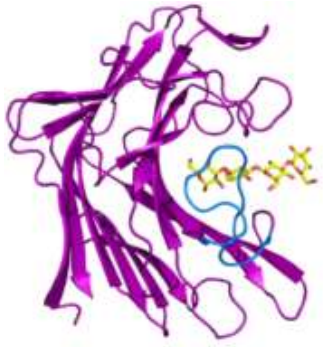
CNRS UPMC
Station Biologique
Roscoff

UPMC
SORBONNE UNIVERSITÉS

Treasure hunting in the genome of the marine bacterium *Zobellia galactanivorans*:

Discovery of novel enzymes for the conversion of algal polysaccharides





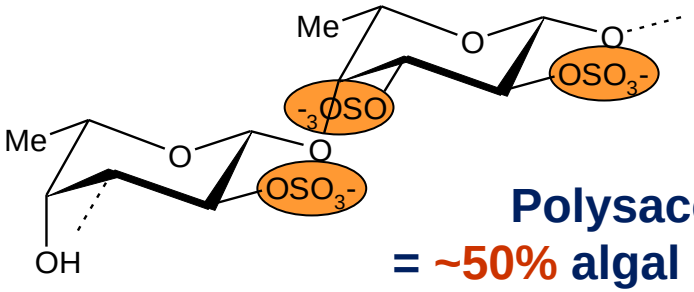
Gurvan Michel

Station Biologique de Roscoff

Marine Glycobiology group

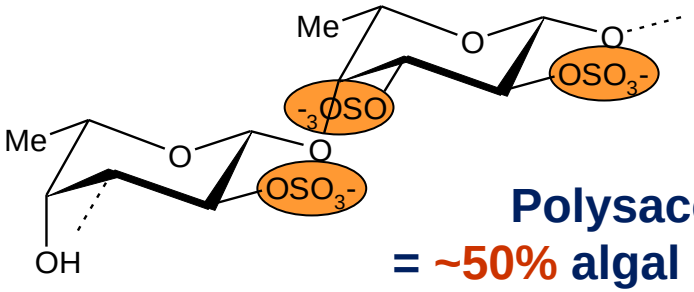
UMR 8227

Macroalgae: crucial role in the coastal primary production



Polysaccharides
= ~50% algal biomass

Macroalgae: crucial role in the coastal primary production



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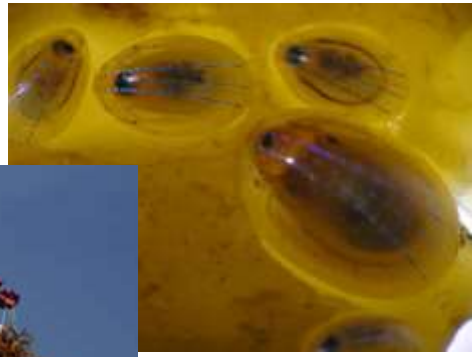


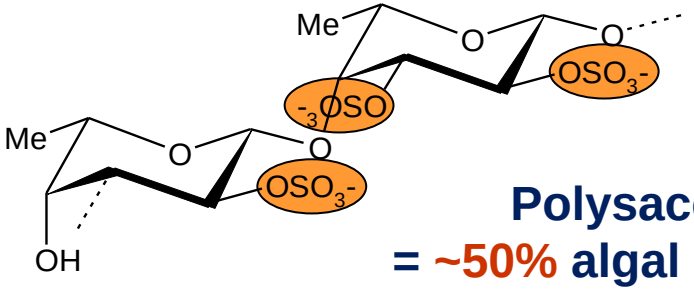
Support the coastal food webs:

from marine herbivorous animals

to human activities

(sea vegetables, hydrocolloids)

Macroalgae: crucial role in the coastal primary production



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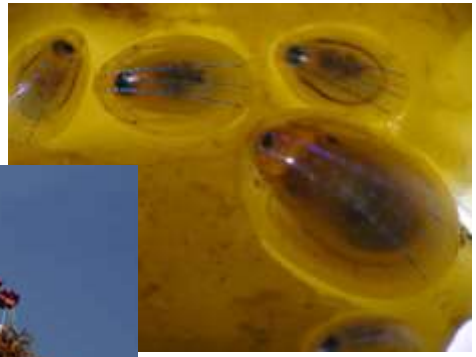
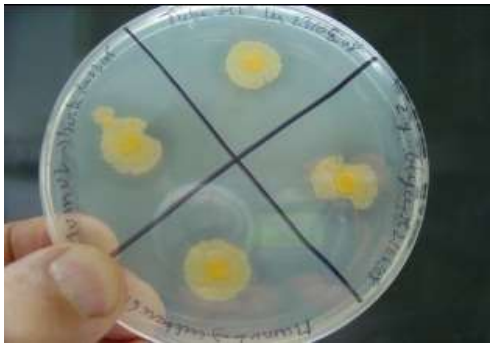
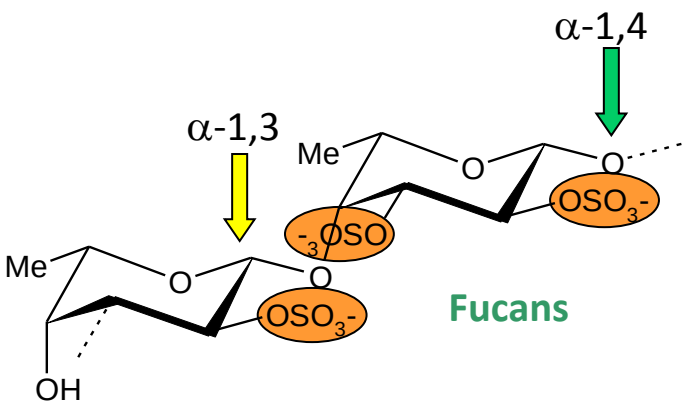




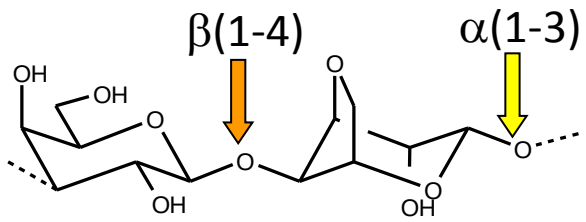
Marine heterotrophic bacteria:

key players in the recycling of algal biomass

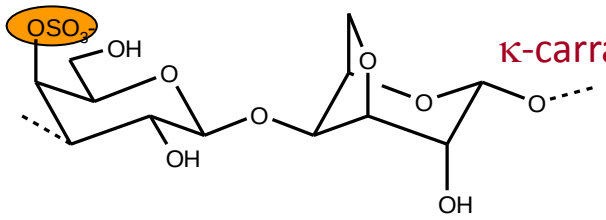
Algal polysaccharides: a underexploited renewable biomass



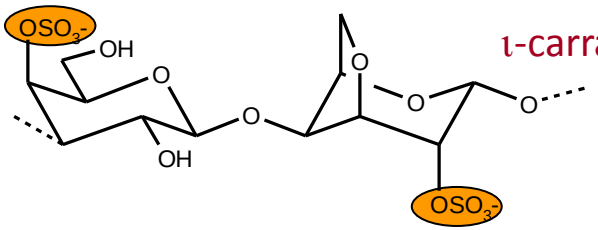
Fucans



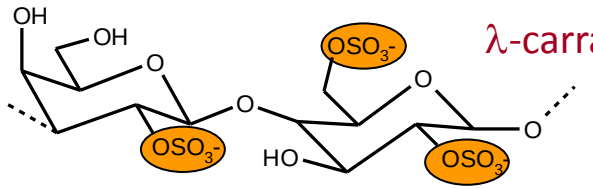
agarose



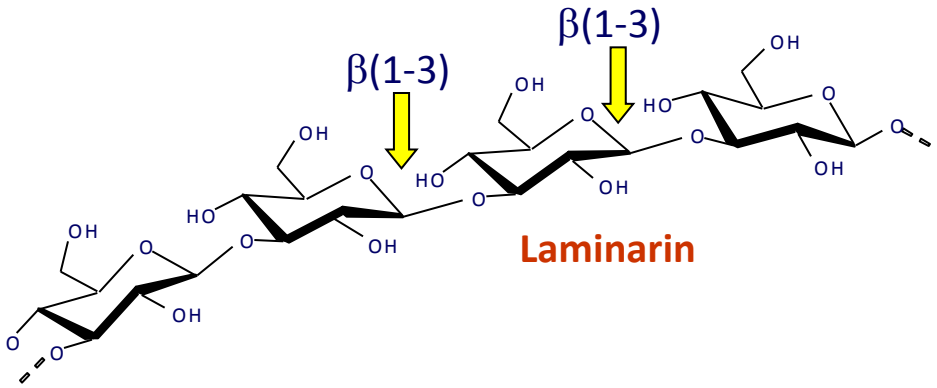
κ-carrageenan



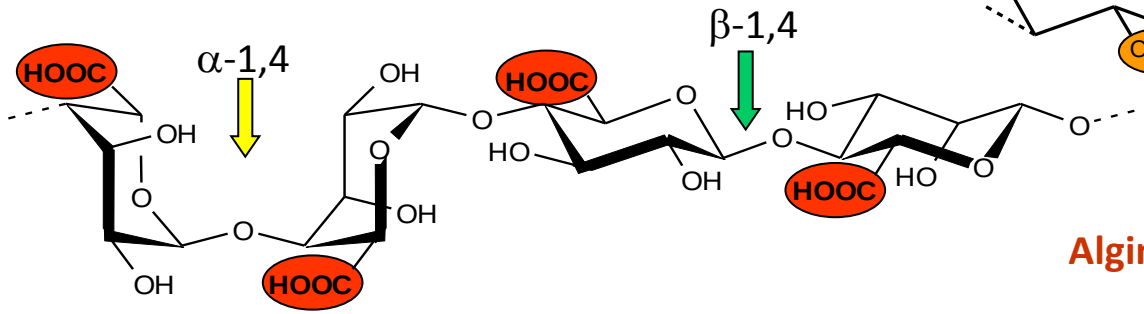
ι-carrageenan



λ-carrageenan



Laminarin

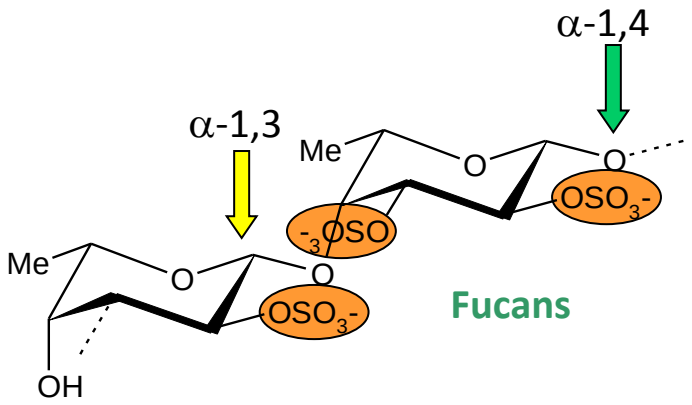


Alginates

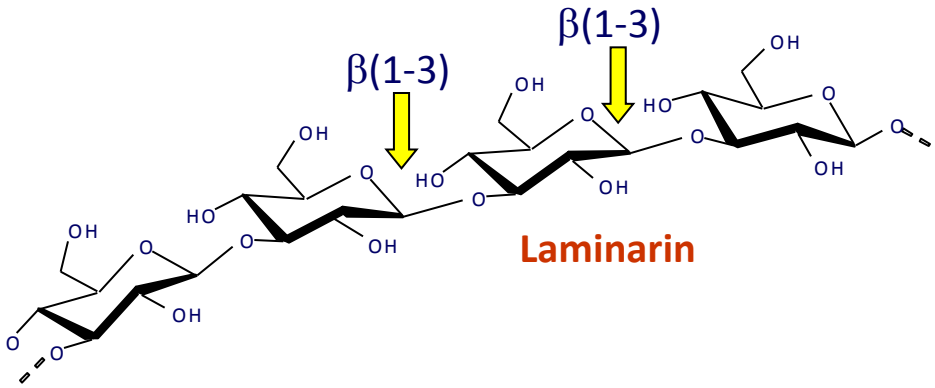
L-Guluronic acids

D-Mannuronic acids

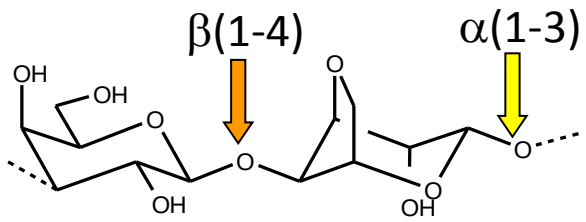
Algal polysaccharides: a underexploited renewable biomass



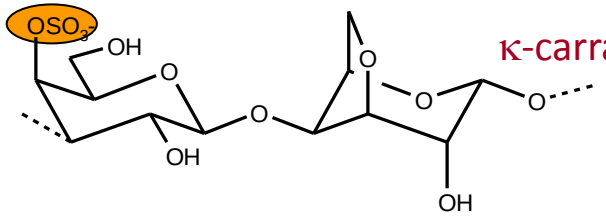
Fucans



Laminarin



agarose



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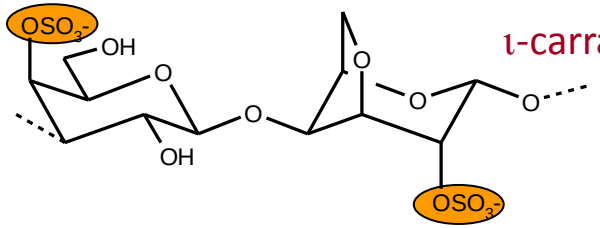

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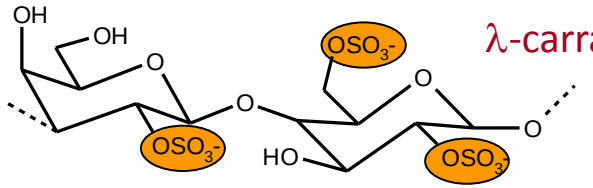


λ-carrageenan

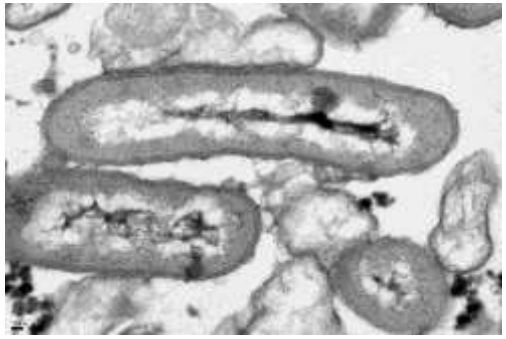
Huge chemical diversity

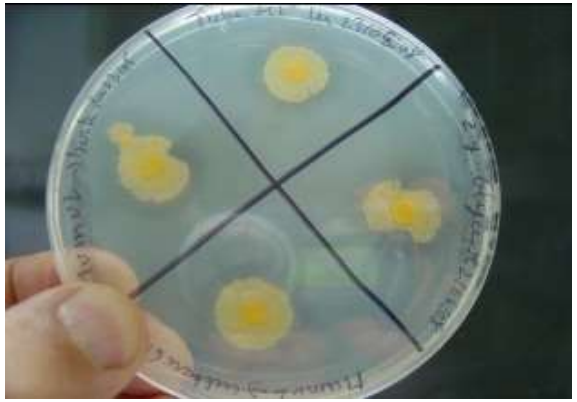
→ Algal sulfated polysaccharides have no equivalent in land plants

→Diversity of the enzymes involved in their biosynthesis and their biodegradation

Zobellia galactanivorans: a model marine bacterium for algae-bacteria interactions

- ***Bacteroidetes*** isolated in Roscoff on a red alga
- Degrade **most algal polysaccharides**





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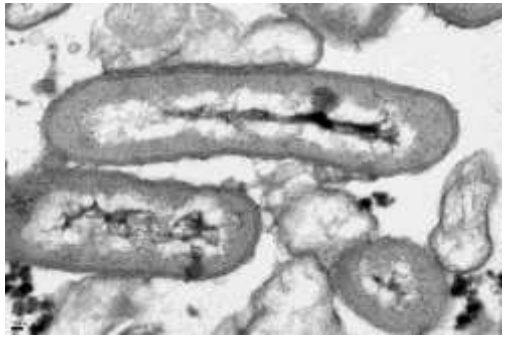

Genome sequencing (4738 genes, 5.5 MB)

- Large system for **substrate detection** and **import**
  - **119** TonB-dependent receptors (TBDR) and **65** one/two-component systems
- Confirmation of the huge potential for **polysaccharide degradation**:
  - **141** Glycoside hydrolases (GH) and **71** sulfatases !

GENOSCOPE
Centre National de Séquençage

Zobellia galactanivorans: a model marine bacterium for algae-bacteria interactions

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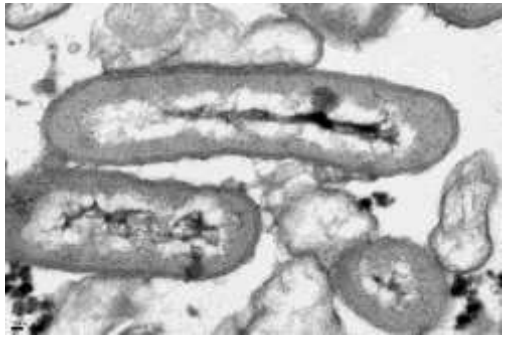

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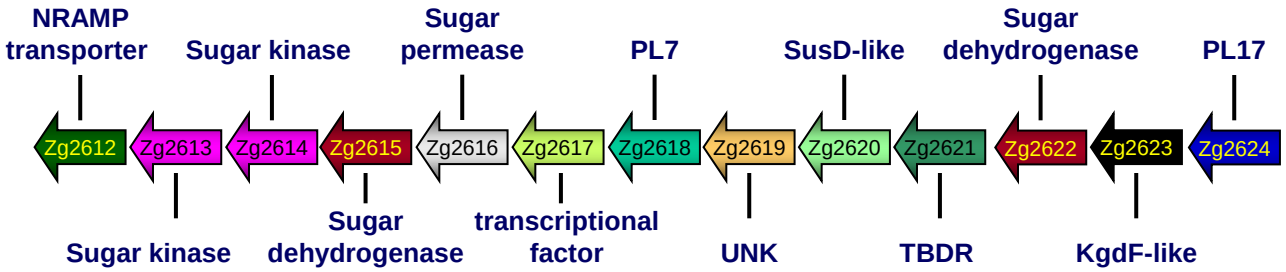
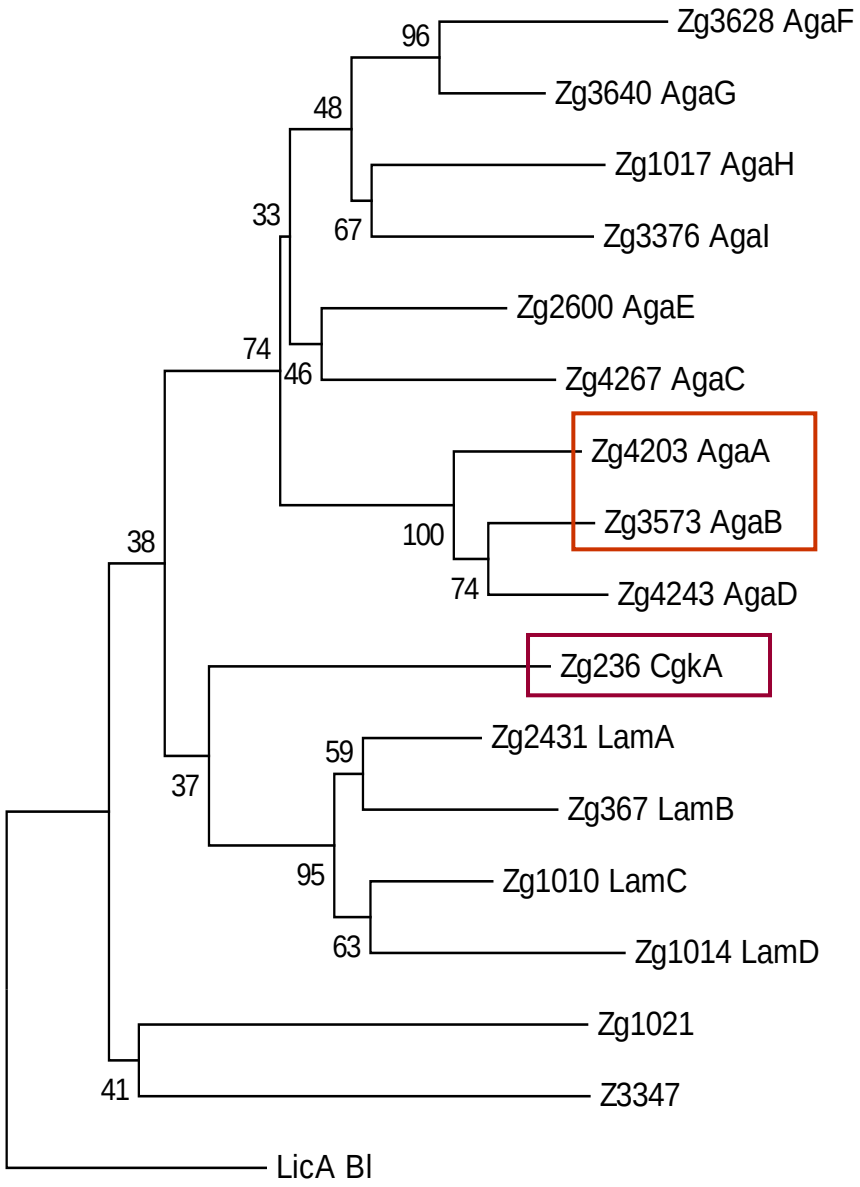
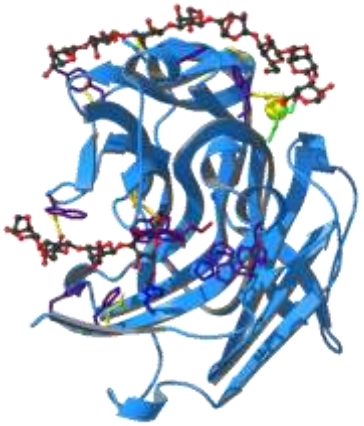
  - ➢ **136** Glycoside hydrolases (GH) and **71** sulfatases !
- Numerous putative **operons** specific for polysaccharide utilization



Phylogenetic tree of the GH16 family from *Zobellia*

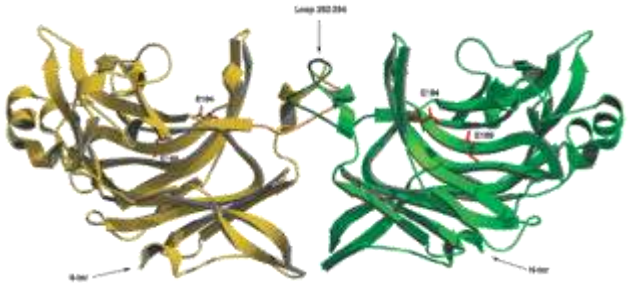


AgaA



Allouch et al, (2003) JBC & (2004) Structure

AgaB



Jam et al (2005) Biochem J

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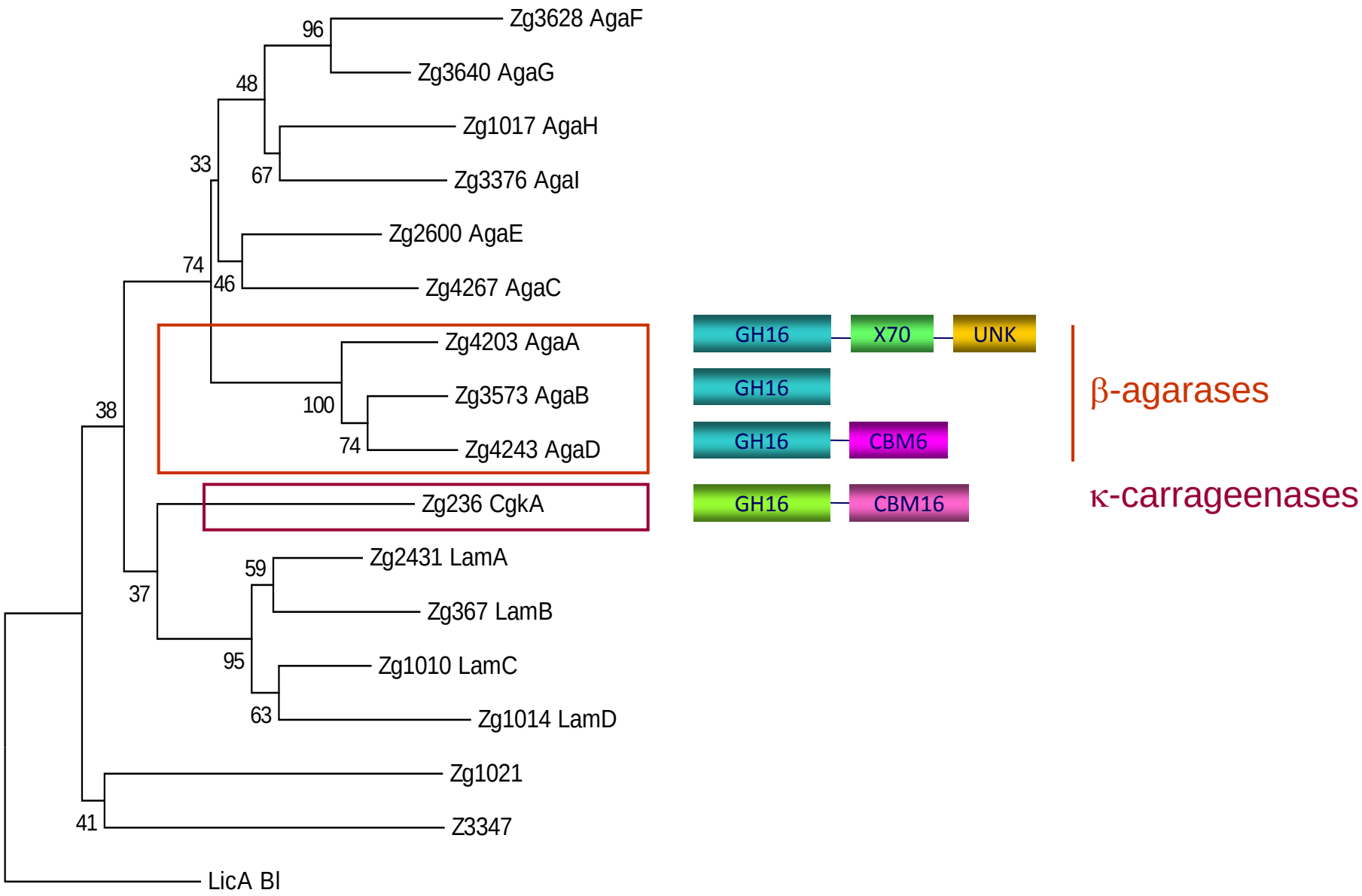

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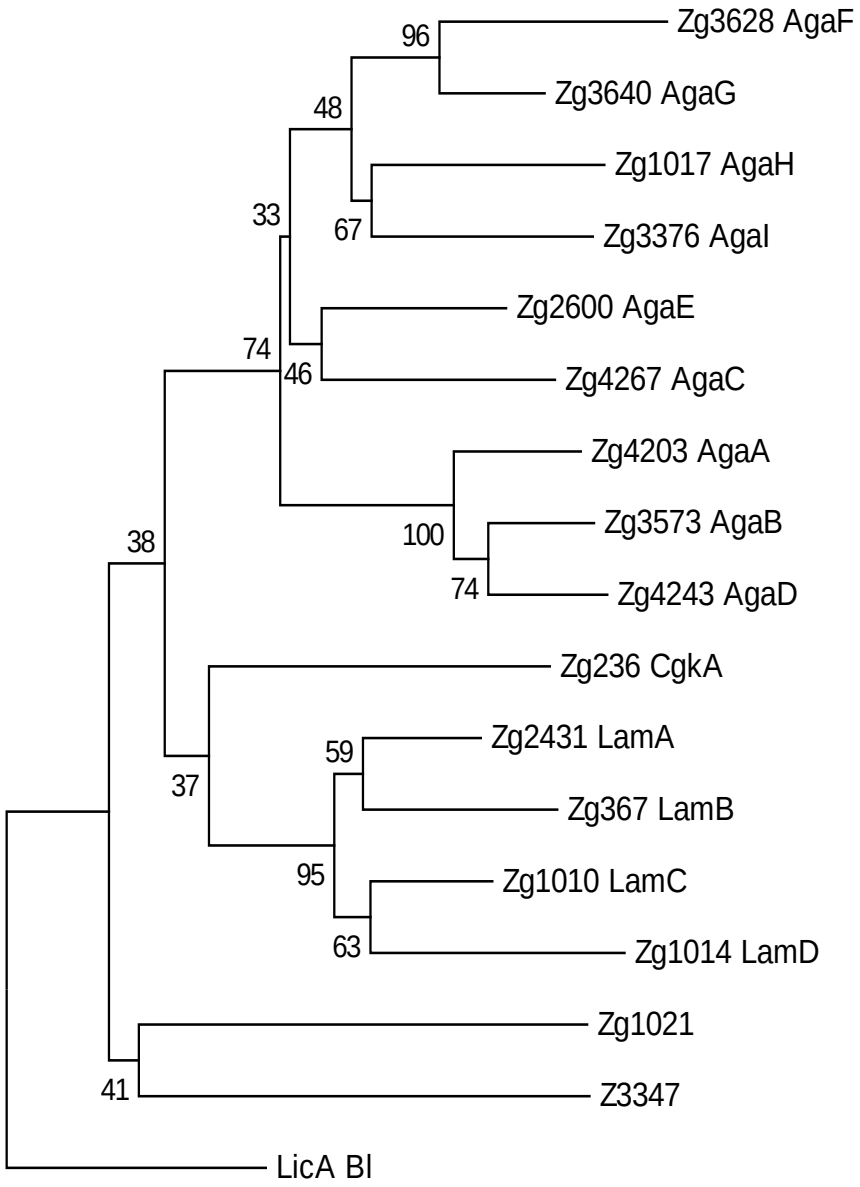

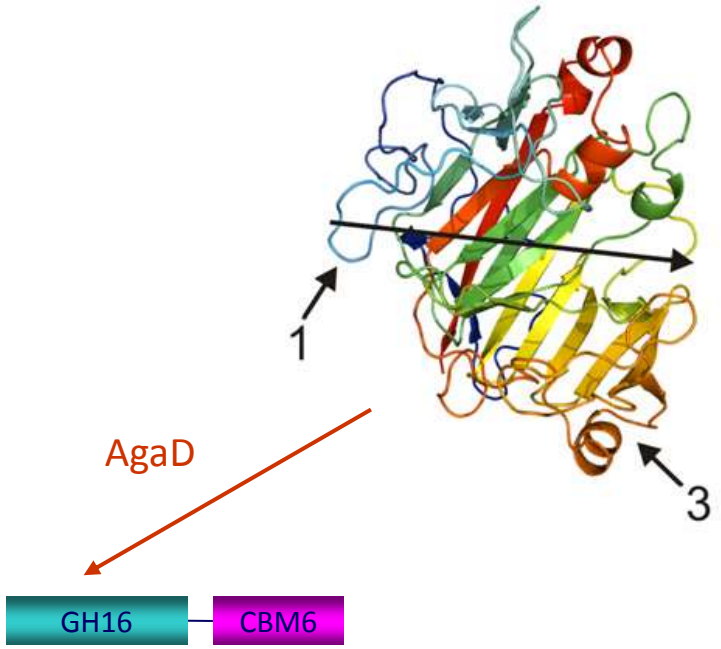

Hehemann et al (2013) JBC

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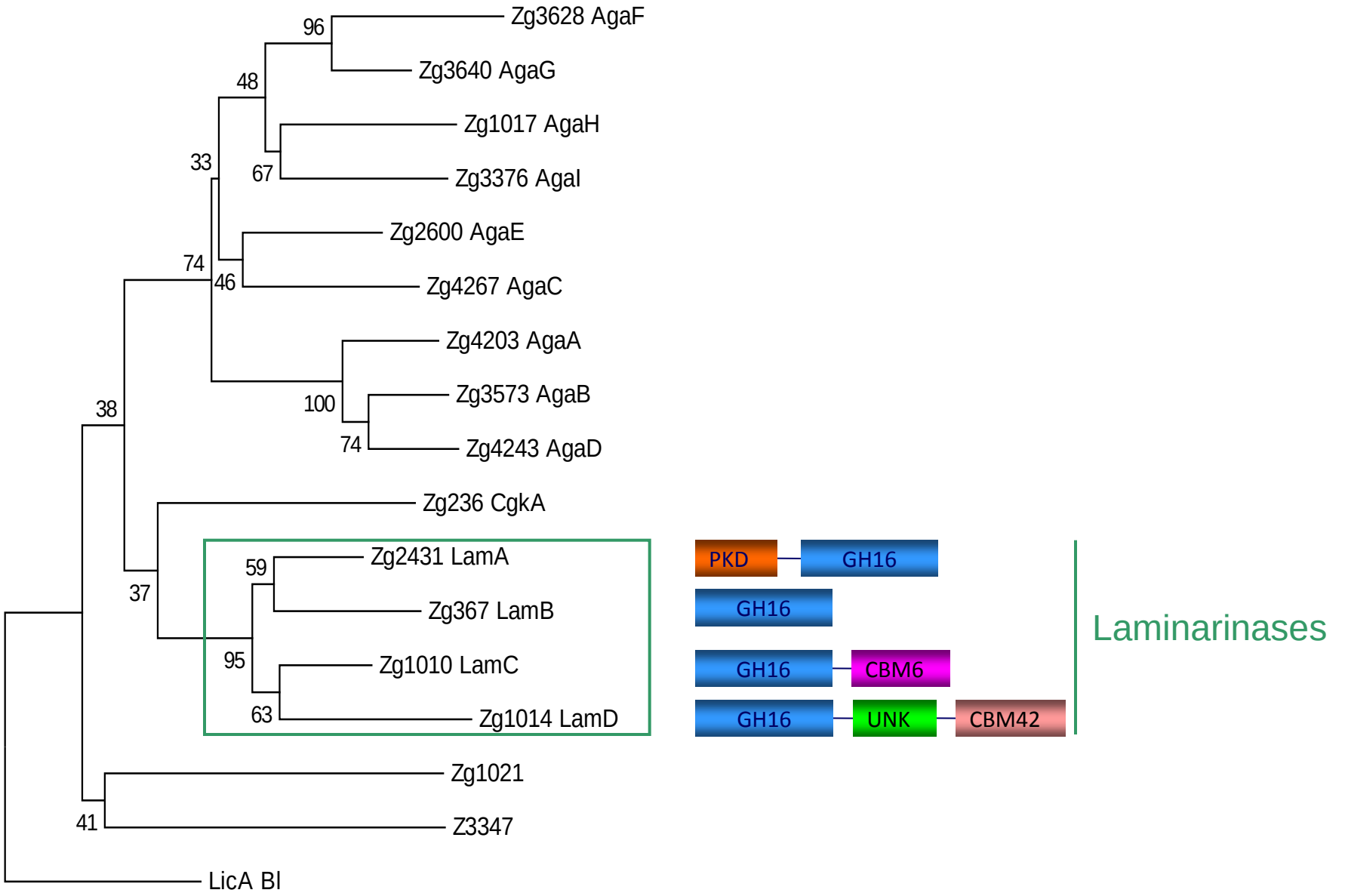

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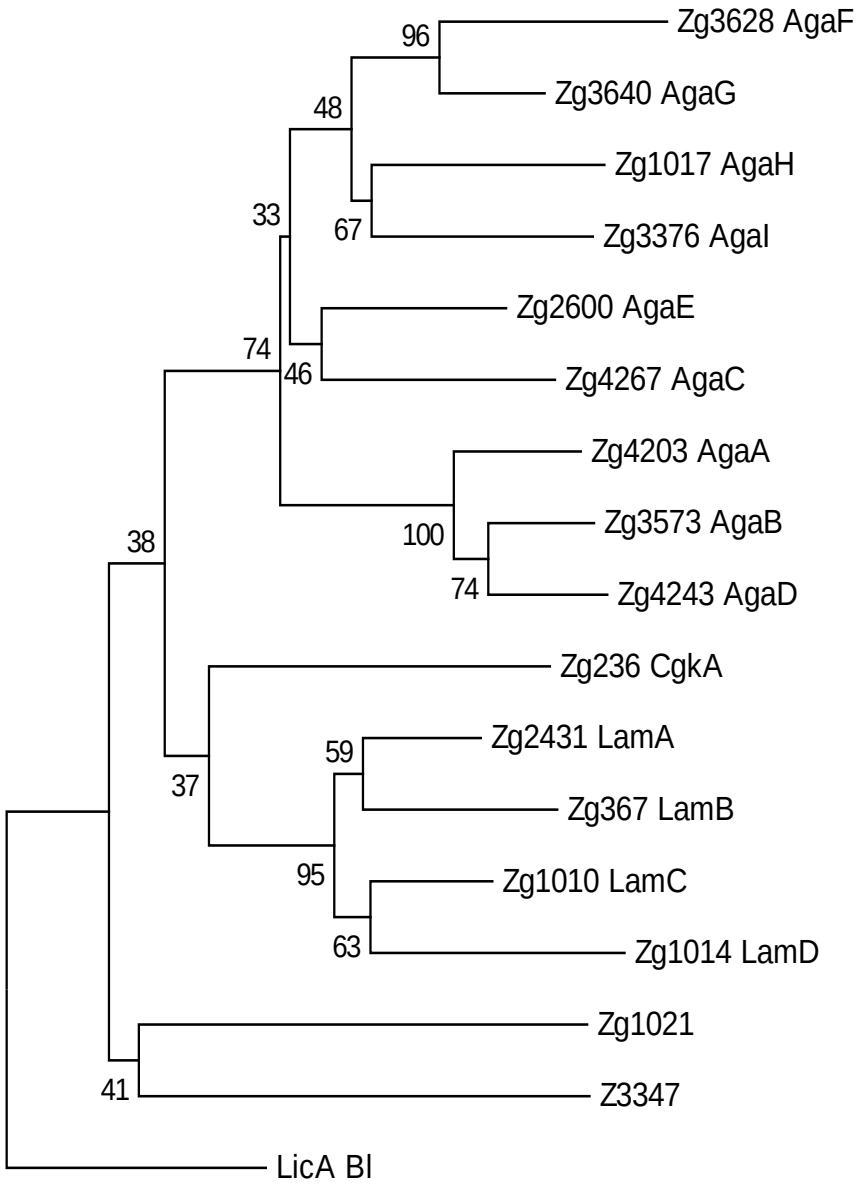
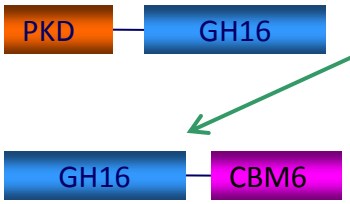


Labourel et al (2014) JBC

LamA_*GH16*

LamC_*GH16*



LamC_*CBM6*

Labourel et al (2014) Acta Cryst D

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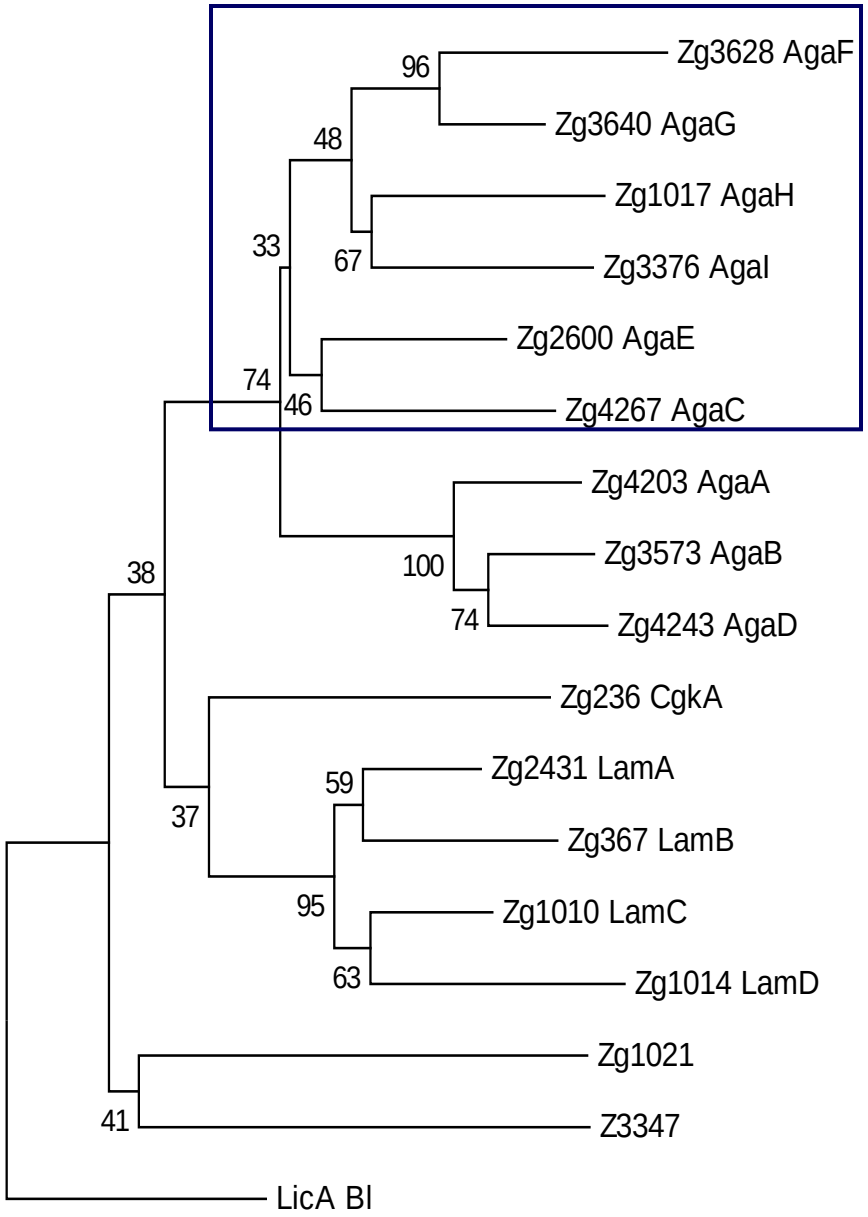

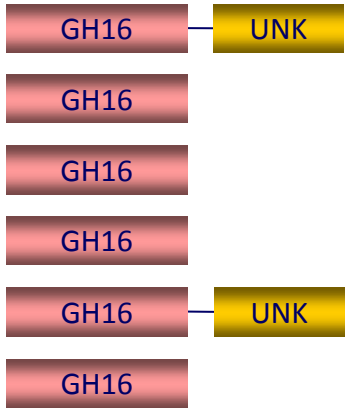

A new sub-family of GH16 ?

~25% identity with AgaA and CgkA

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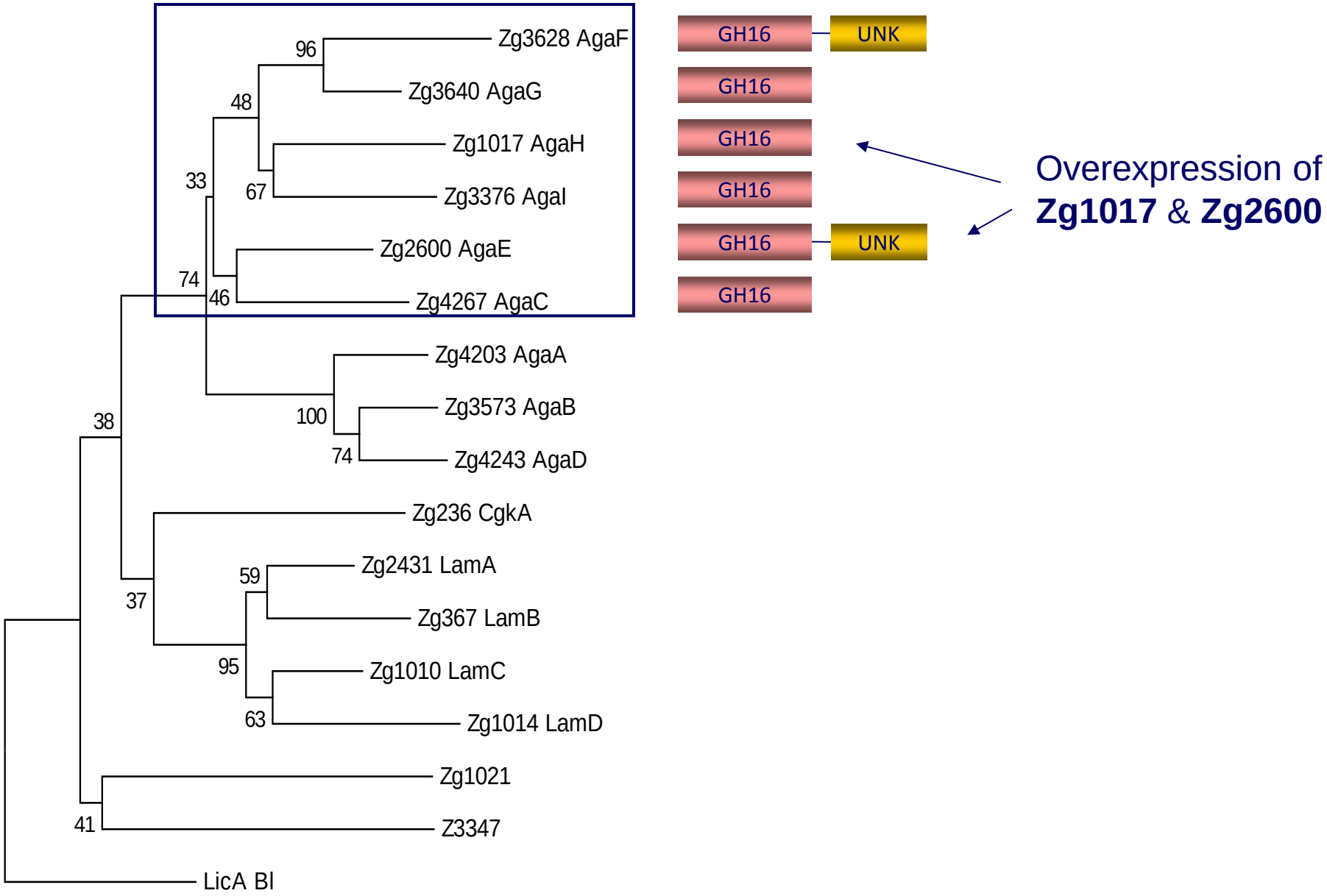
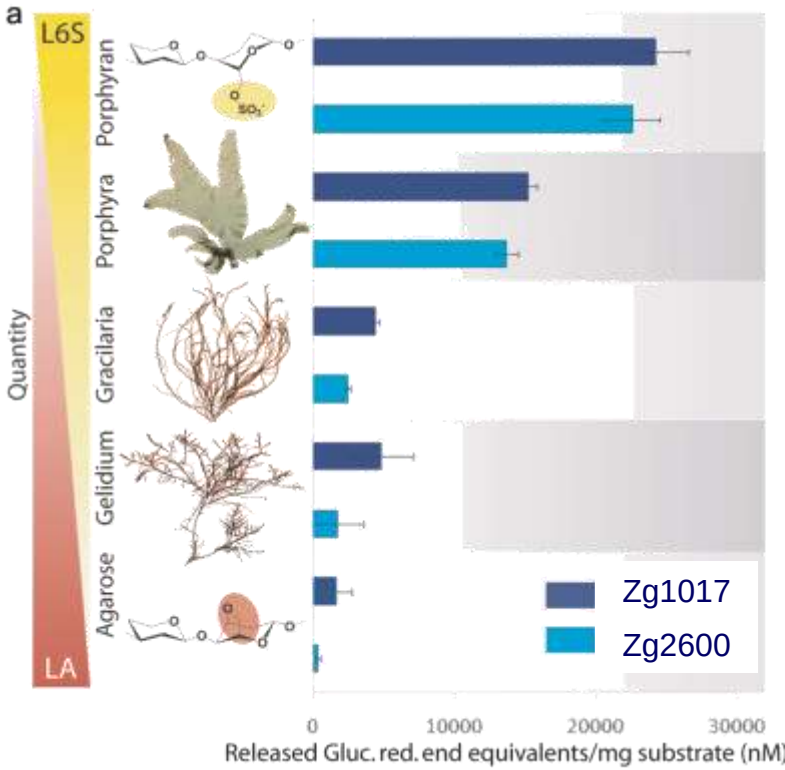


Activity screening on cell wall extracts from seaweeds



- Inactive on agarose and carrageenans

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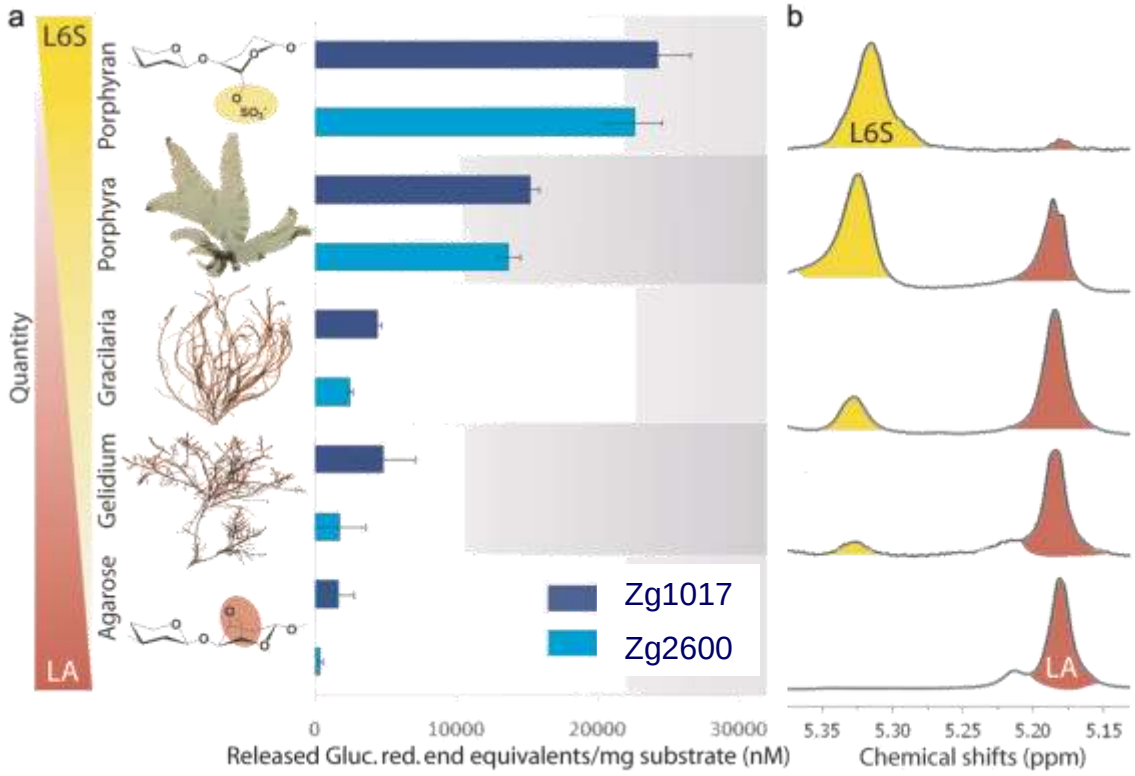
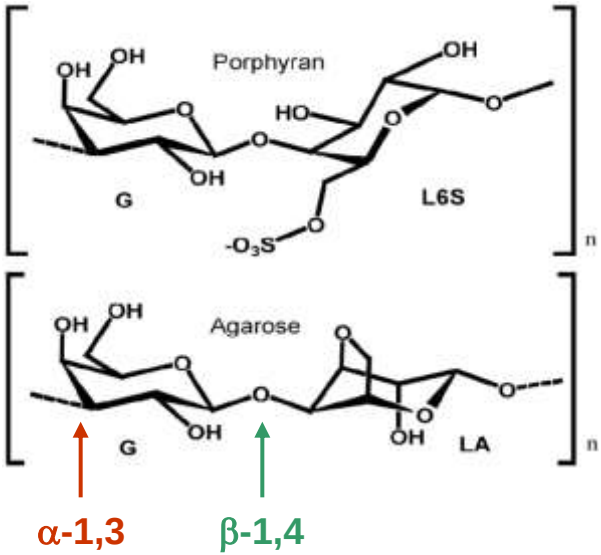


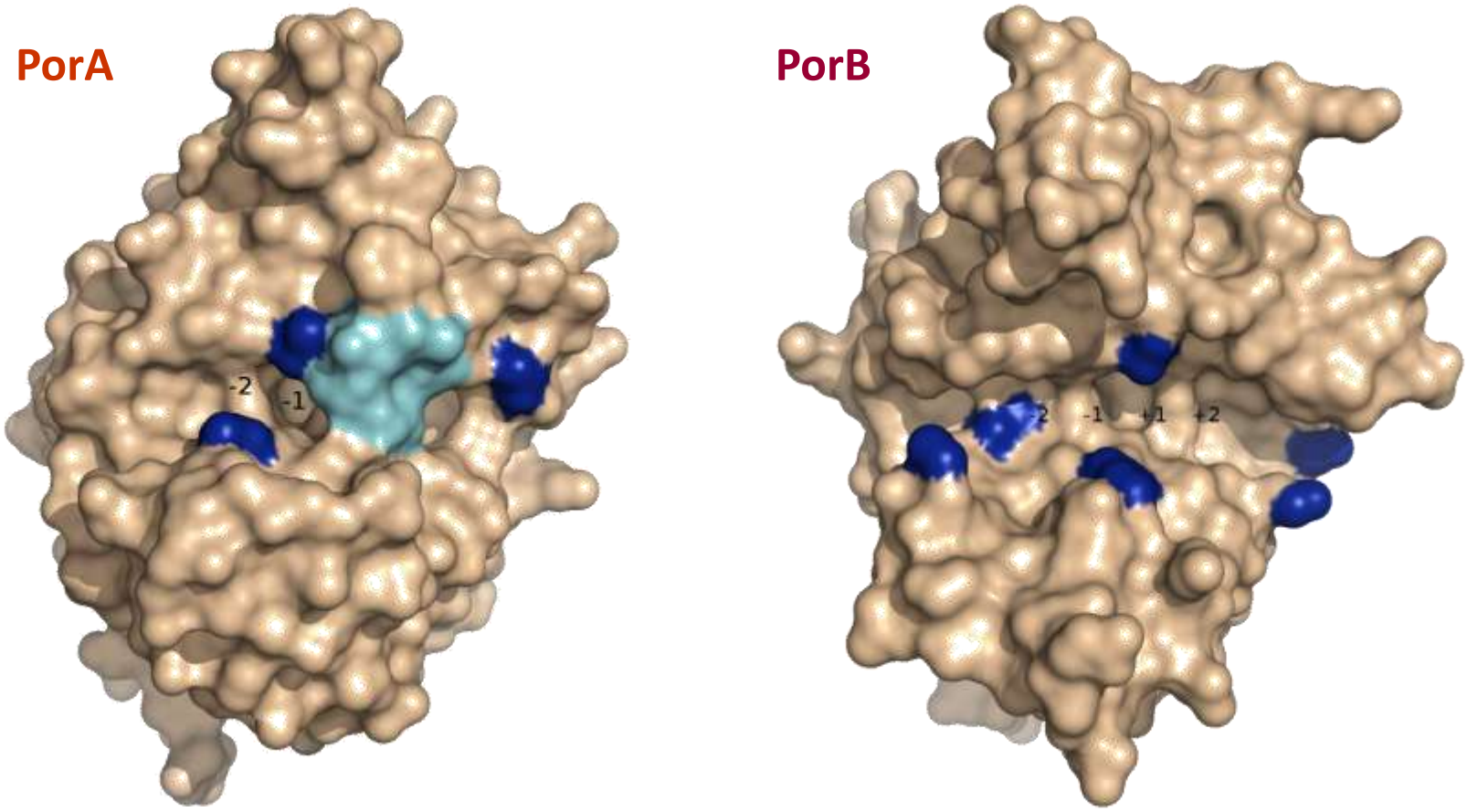
L6S = L-galactose-6-sulfate
LA = 3,6-anhydro-L-galactose



- Inactive on agarose and carrageenans
- Main end product: Porphyran disaccharide (L6S-G)
- Hydrolysis of beta-1,4 glycosidic linkage

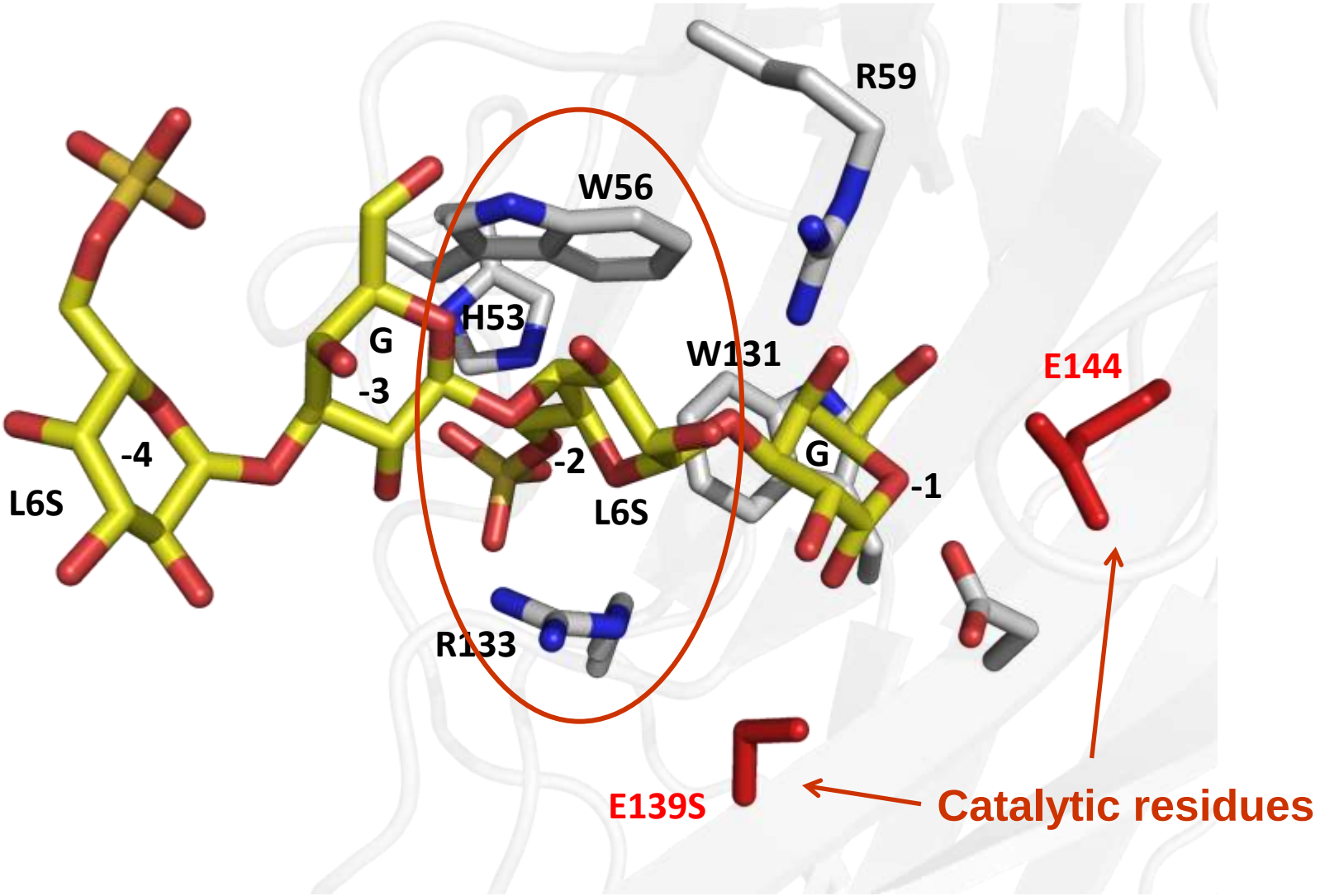
Zg2600 (PorA) et Zg1017 (PorB) are the first beta-porphyranases

Crystal structure of the β-porphyranases PorA and PorB



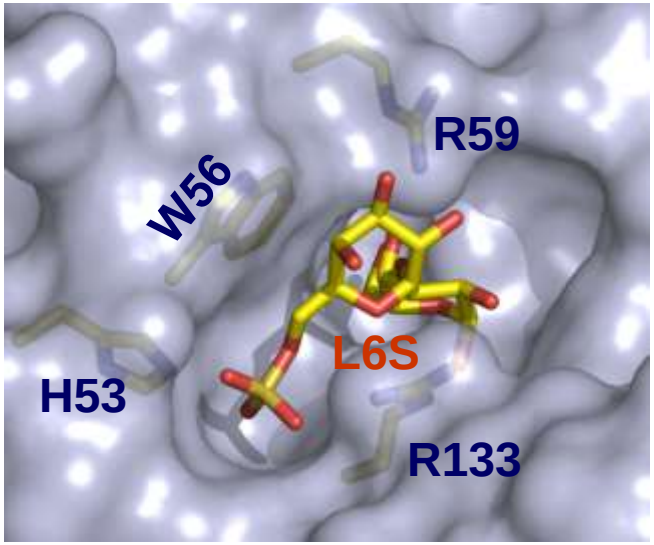
Molecular surface (Basic residues are colored in dark blue)

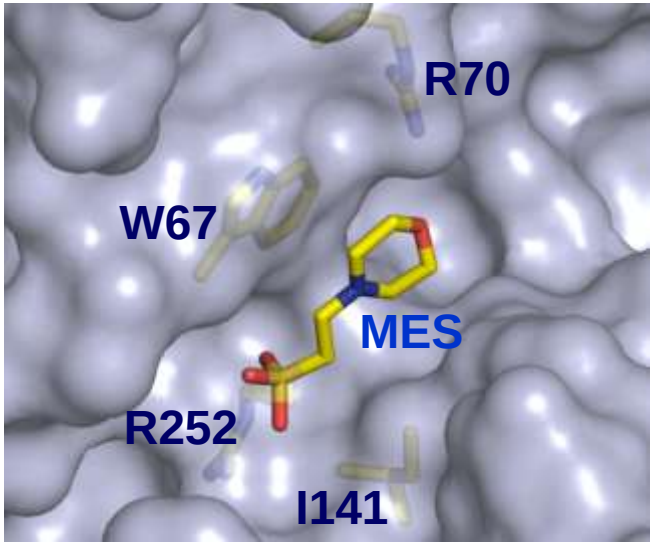
Structure of PorA in complex with porphyran tetrasaccharide



Conserved basic residues at subsite -2 are critical for porphyran recognition

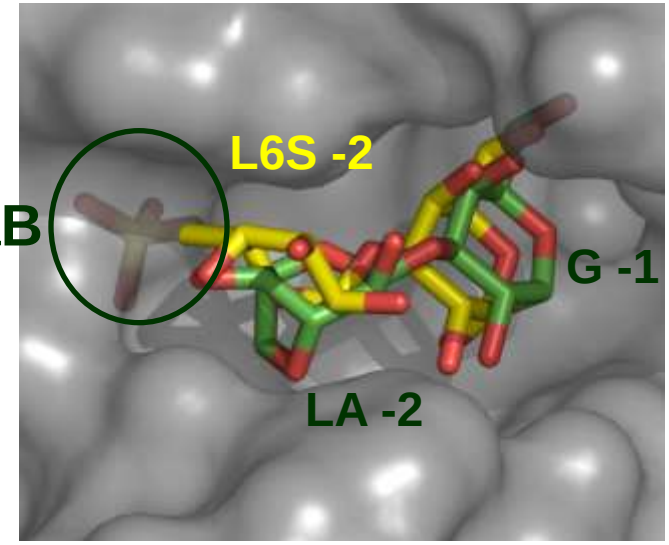
PorA





PorB

AgaB



No pocket in β-agarases:

Steric clash with L6S at subsite -2

→ agarases cannot degrade porphyran

Hehemann et al (2010) Nature

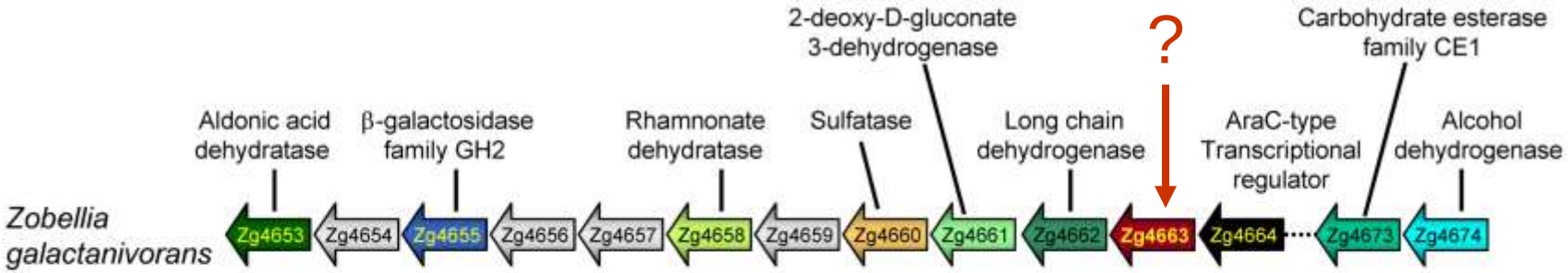
Discovery of a new GH family in *Zobellia*

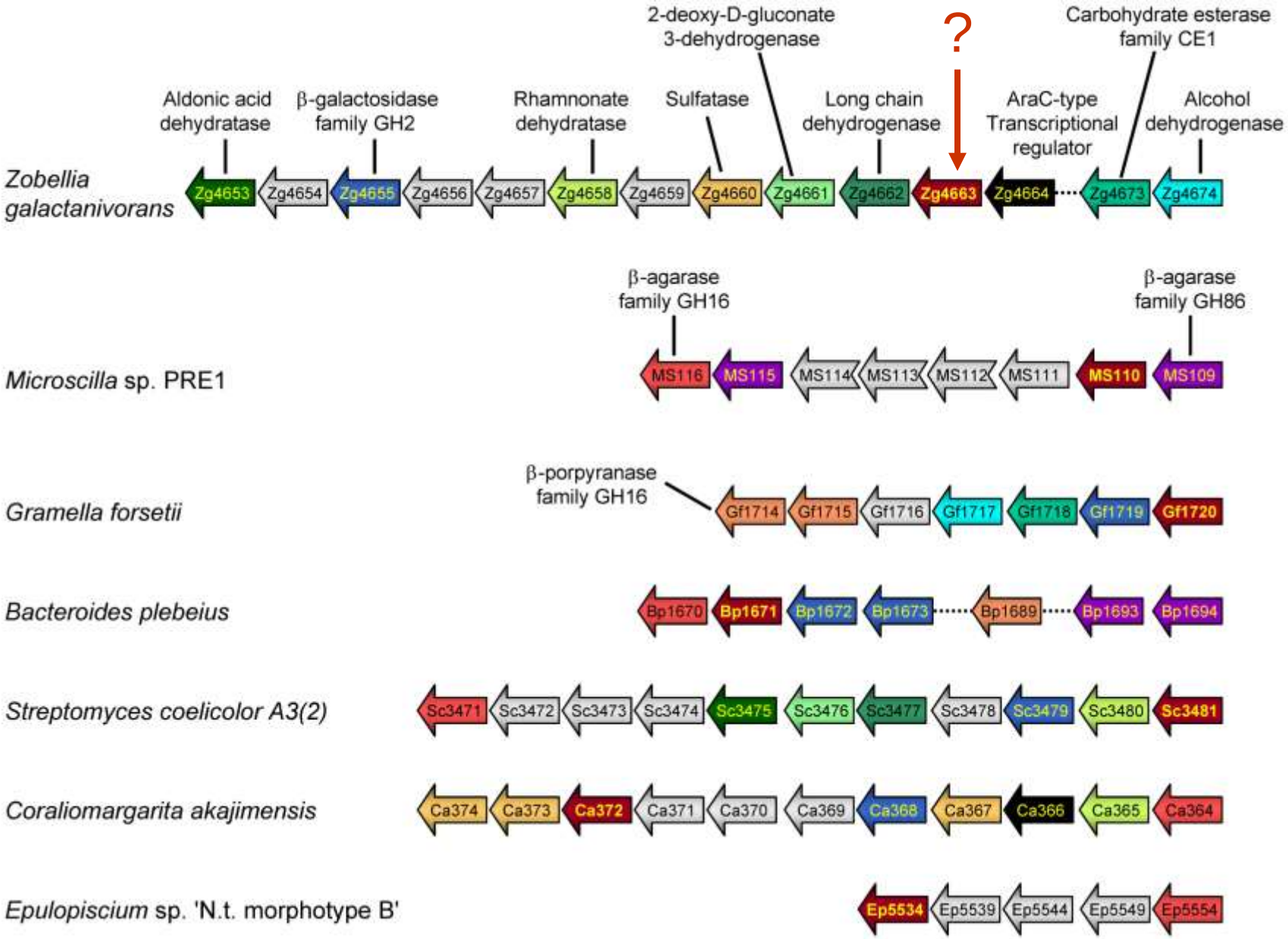
- 5 paralogous proteins distantly related to GH43 (~15% sequence identity)
- Always localized in gene clusters with CAZymes and sulfatases

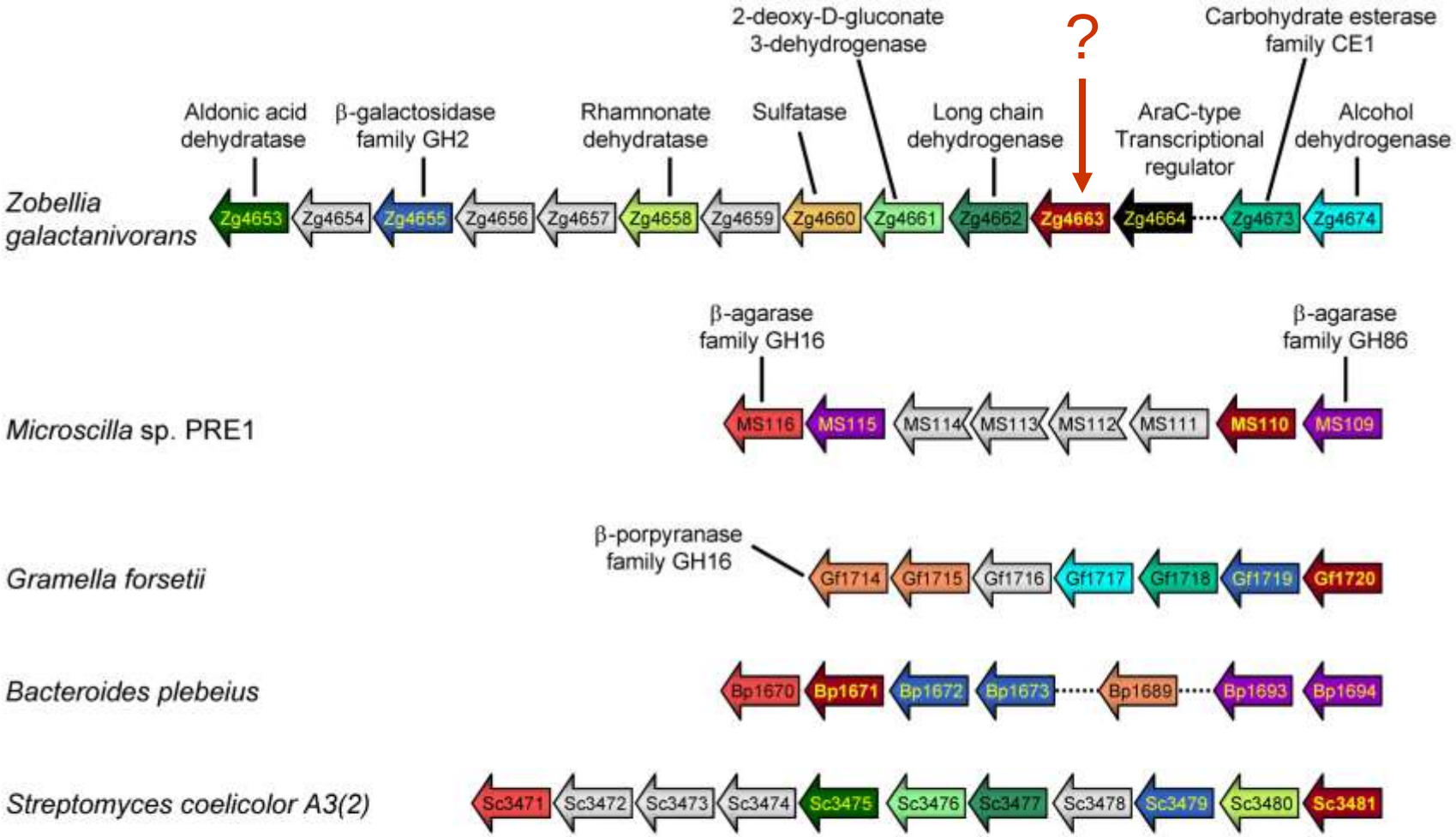
Hypothesis: **new GH** specific for **algal sulfated polysaccharides**?

- Zg3597 and Zg4663: soluble expressed in the MARINE-EXPRESS project (Groisillier et al, 2010, Molecular Cell Factories)
- Purification and crystallization of Zg3597 and Zg4663
- Reducing sugar activity screening on our collection of algal polysaccharides and cell wall extracts

Unsuccessful…

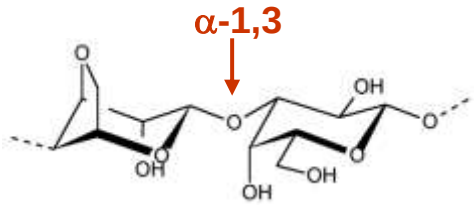

2-deoxy-D-gluconate 3-dehydrogenase
?
Carbohydrate esterase family CE1
Aldonic acid dehydratase
β-galactosidase family GH2
Rhamnonate dehydratase
Sulfatase
Long chain dehydrogenase
AraC-type Transcriptional regulator
Alcohol dehydrogenase
Zobellia galactanivorans
Zg4653
Zg4654
Zg4655
Zg4656
Zg4657
Zg4658
Zg4659
Zg4660
Zg4661
Zg4662
Zg4663
Zg4664
Zg4673
Zg4674


2-deoxy-D-gluconate 3-dehydrogenase
?
Carbohydrate esterase family CE1
Aldonic acid dehydratase
β-galactosidase family GH2
Rhamnonate dehydratase
Sulfatase
Long chain dehydrogenase
AraC-type Transcriptional regulator
Alcohol dehydrogenase
Zobellia galactanivorans
Zg4653 Zg4654 Zg4655 Zg4656 Zg4657 Zg4658 Zg4659 Zg4660 Zg4661 Zg4662 Zg4663 Zg4664 Zg4673 Zg4674
β-agarase family GH16
β-agarase family GH86
Microscilla sp. PRE1
MS116 MS115 MS114 MS113 MS112 MS111 MS110 MS109
β-porpyranase family GH16
Gramella forsetii
Gf1714 Gf1715 Gf1716 Gf1717 Gf1718 Gf1719 Gf1720
Bacteroides plebeius
Bp1670 Bp1671 Bp1672 Bp1673 Bp1689 Bp1693 Bp1694
Streptomyces coelicolor A3(2)
Sc3471 Sc3472 Sc3473 Sc3474 Sc3475 Sc3476 Sc3477 Sc3478 Sc3479 Sc3480 Sc3481
Coraliomargarita akajimensis
Ca374 Ca373 Ca372 Ca371 Ca370 Ca369 Ca368 Ca367 Ca366 Ca365 Ca364
Epulopiscium sp. 'N.t. morphotype B'
Ep5534 Ep5539 Ep5544 Ep5549 Ep5554



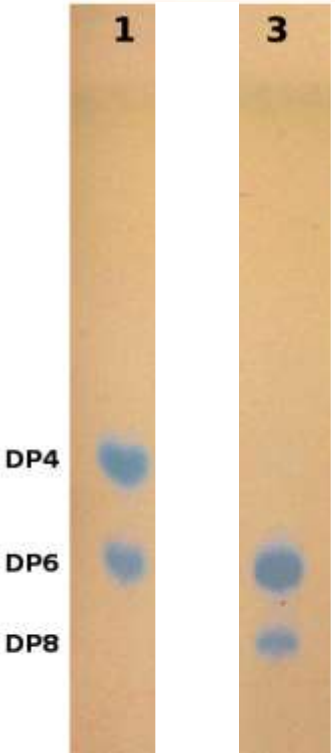
Surrounded by β-agarases, β-porphyranases and β-galactosidases

→ Zg4663: a **α-1,3-galactosidase** specific for agarocolloids?

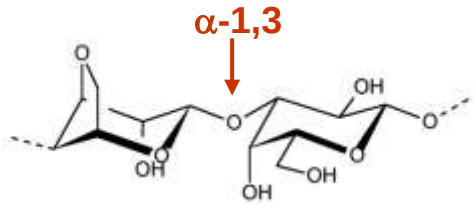


Neoagarobiose (DP2)

Test of the activity of Zg4663 on oligo-agars released by the β-agarase AgaB



Thin layer chromatography



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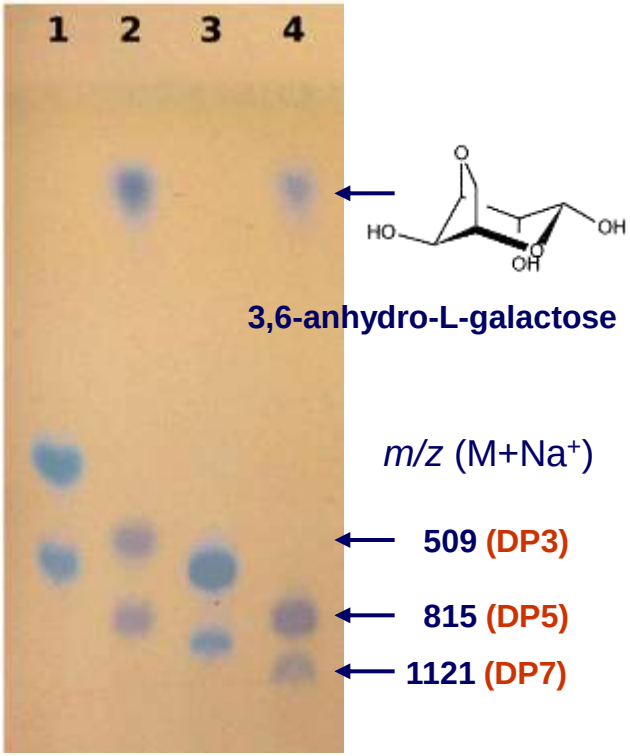


3,6-anhydro-L-galactose

Thin layer chromatography

m/z (M+Na$^+$)

509 (DP3)

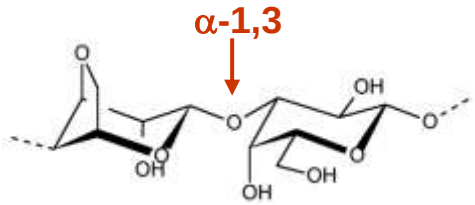
815 (DP5)

1121 (DP7)

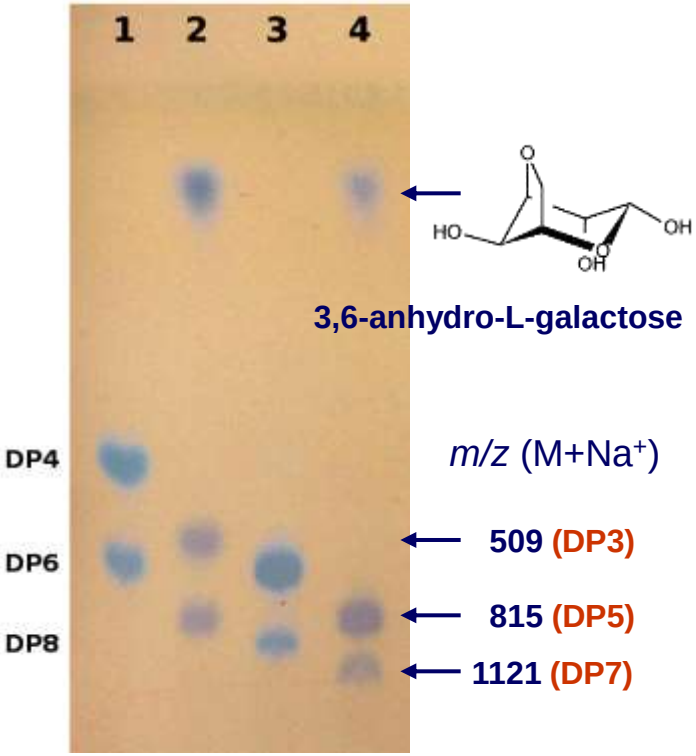
Lane 2 and 4: addition of **Zg4663**

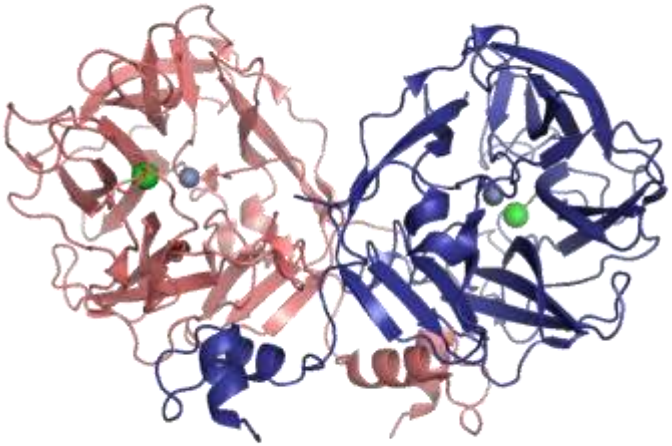
Size of the products determined by mass spectrometry (MALDI-TOF)

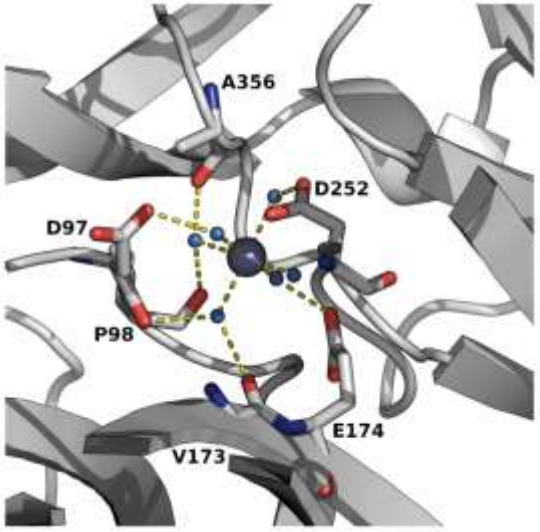
- Zg4663 is a **1,3-α 3,6-anhydro-L-galactosidase** (last step of agar degradation)



Neoagarobiose (DP2)







- Zg4663 is a **1,3-α 3,6-anhydro-L-galactosidase** (last step of agar degradation)
- Adopts a five-bladed β-propeller fold and forms a dimer by domain swapping
- Displays a zinc-dependent catalytic machinery

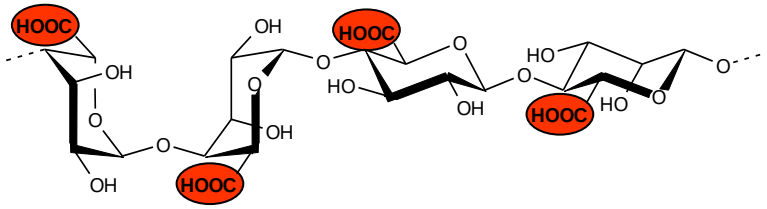
Rebuffet et al (2011) Environmental Microbiology

Zobellia genome contains

7 putative alginate lyases

	Alginate lyases						
CAZy family	PL5	PL6	PL7	PL14	PL15	PL17	PL18
Z. galactanivorans enzymes	-	AlyA4 AlyA6	AlyA1 AlyA2 AlyA5	AlyA7	Zg4327	AlyA3	-

Different specificities?

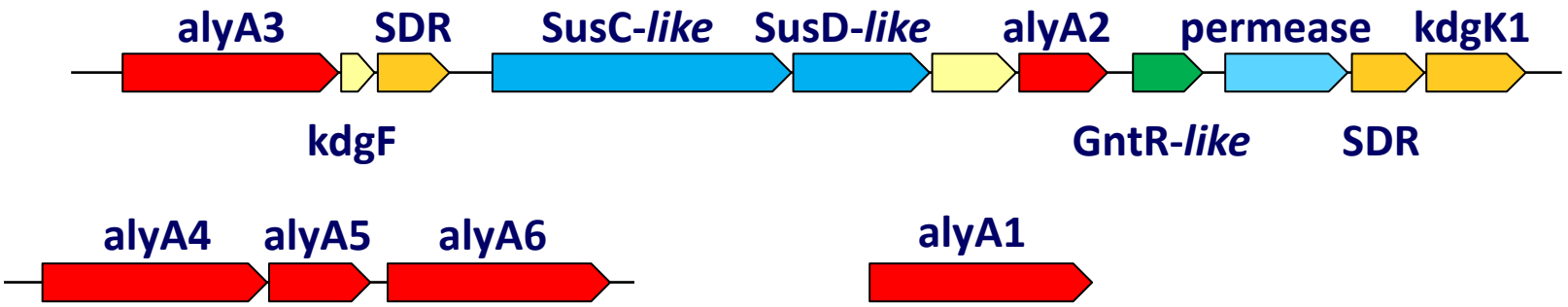


L-Guluronic acids D-Mannuronic acids

Different modes of action?

Different biological functions?

Alginate-related gene clusters?



Degradation

Alginate

↓ **Alginate lyases**

4-deoxy-5-keto uronic acid

↓ **Gluconate dehydrogenases (SDR)**

2-keto-3-deoxy-gluconate

↓ **kdgK**

ketodeoxyphosphogluconate

Regulation

GntR-*like* transcription regulator

Transport

Outside

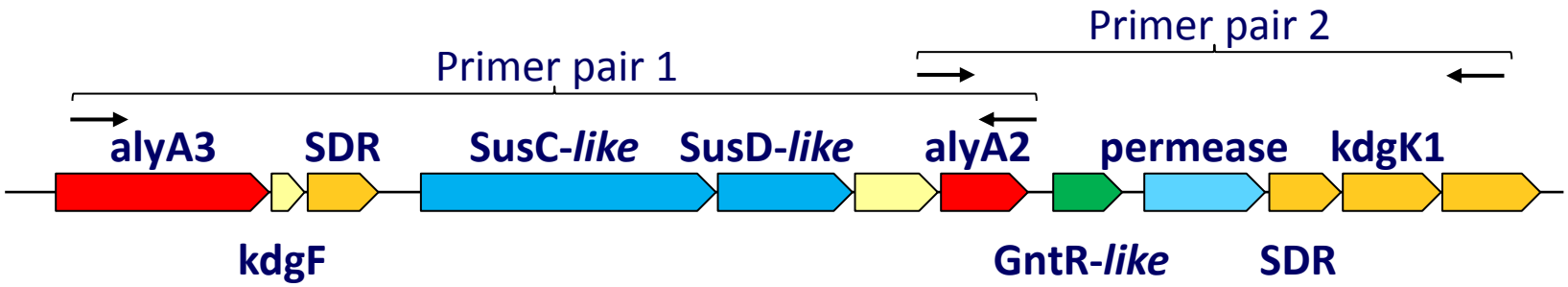
↓ **Sus-*like* system**

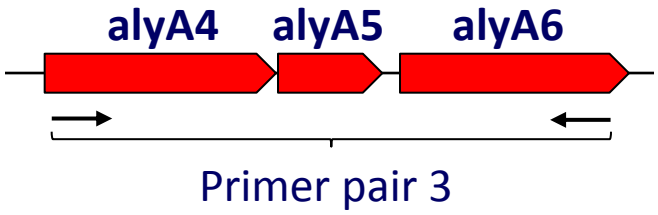
Periplasm

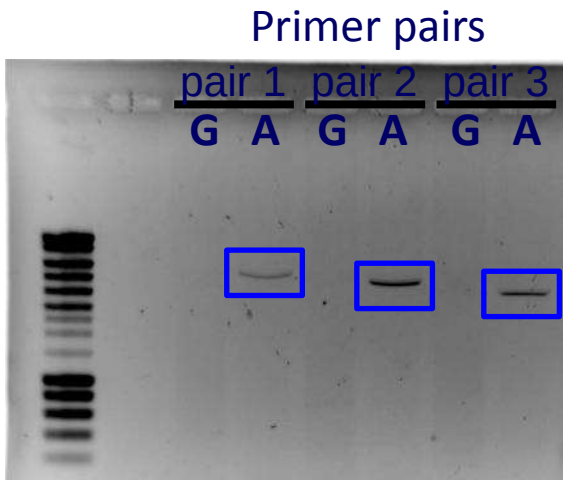
↓ **permease**

Cytoplasm

Identification of two alginolytic operons!

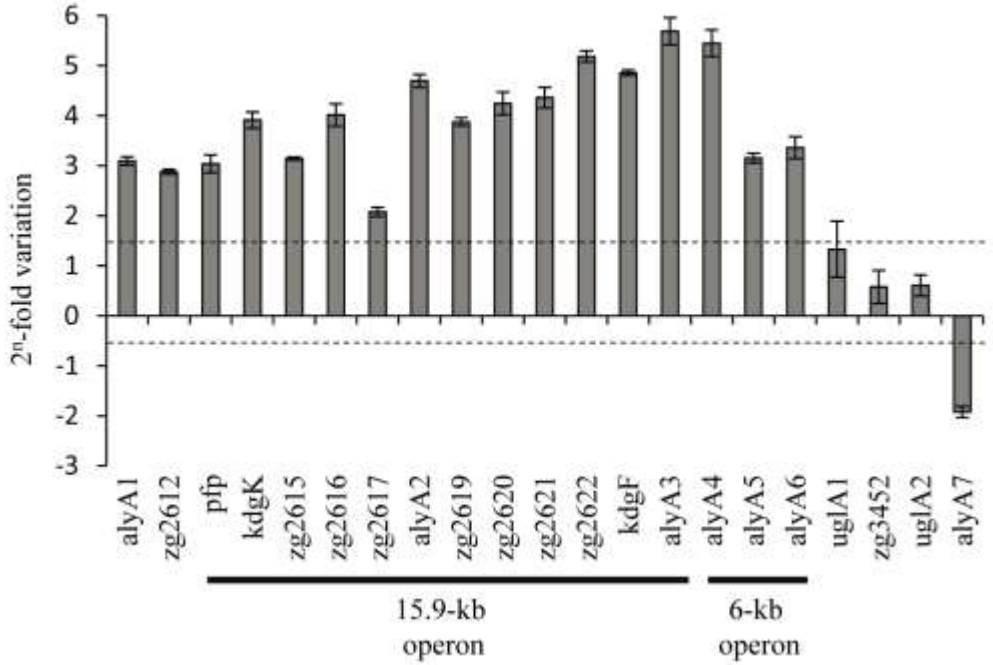






G : glucose A : alginate

Gene expression with alginate vs glucose



Thomas *et al* (2012) Env Microbio

Characterization of alginate-responsive genes

Medium-throughput cloning strategy

Recombinant, soluble proteins

Confirmation of the **enzymatic activity**
for **6 new proteins** from *Z. galactanivorans*

- ***Alginate lyases**: AlyA1, AlyA4, AlyA5 and AlyA7*
- ***2-dehydro-3-deoxygluconokinase:*** Zg2614
- ***2-dehydro-3-deoxy-D-gluconate 6-dehydrogenase:*** Zg2622

Characterization of alginate-responsive genes

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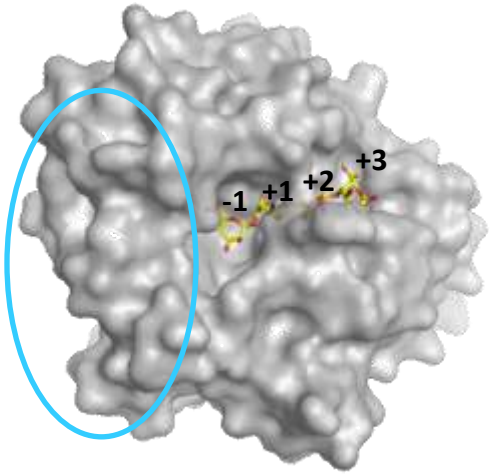
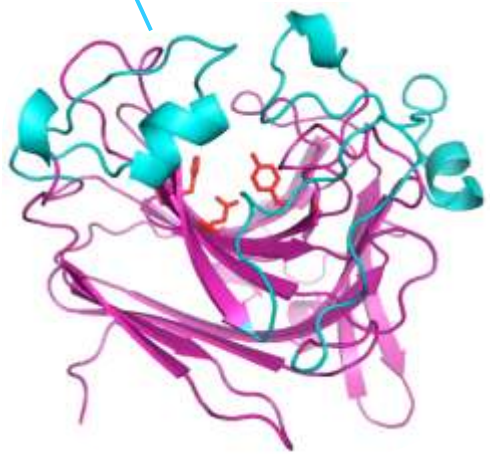
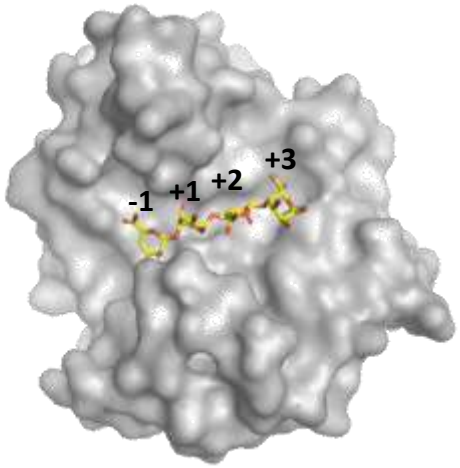
AlyA1 and AlyA5

Two new lyases from the PL7 family





AlyA5: exo-lyase



AlyA1: endo-lyase

Thomas *et al*, (2013) JBC

Conclusions & perspectives

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- **Algae-associated bacteria** are essential sources of novel marine enzymes
- A **(semi)-rational strategy** for the discovery of novel enzymes: combination of phylogenomics, comparative genomics and biochemical approaches
- Importance of **libraries of natural polysaccharides / oligosaccharides** for the screening of real functions

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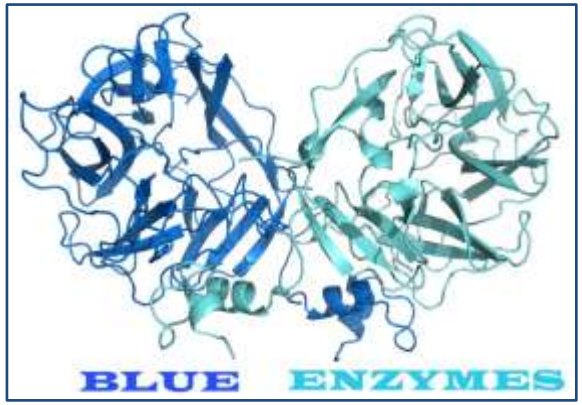
- Importance of **libraries of natural polysaccharides / oligosaccharides** for the screening of real functions

Perspectives: **start of two new projects in 2015**



ALGOLIFE:

processing macroalgae for high value-added human and animal health/nutrition products

Thank you!

Aurore Labourel

Jan-Hendrik Hehemann

François Thomas

Etienne Rebuffet