MICROBIAL REFINING OF ALGAL BIOMASS FOR FOOD AND FEED INGREDIENTS (WP3)

Speaker: Anne S. Meyer DTU

24-03-2021

MacroCascade Final Conference

WP3 Objectives

5 main objectives:

1) Convert algal biomass *anaerobically* into **feed** ingredients by ensiling

2) Convert algal biomass and *rapeseed cake* into **feed** ingredients

3) Develop algae-based *prebiotic* and *probiotic* animal **feed** ingredients

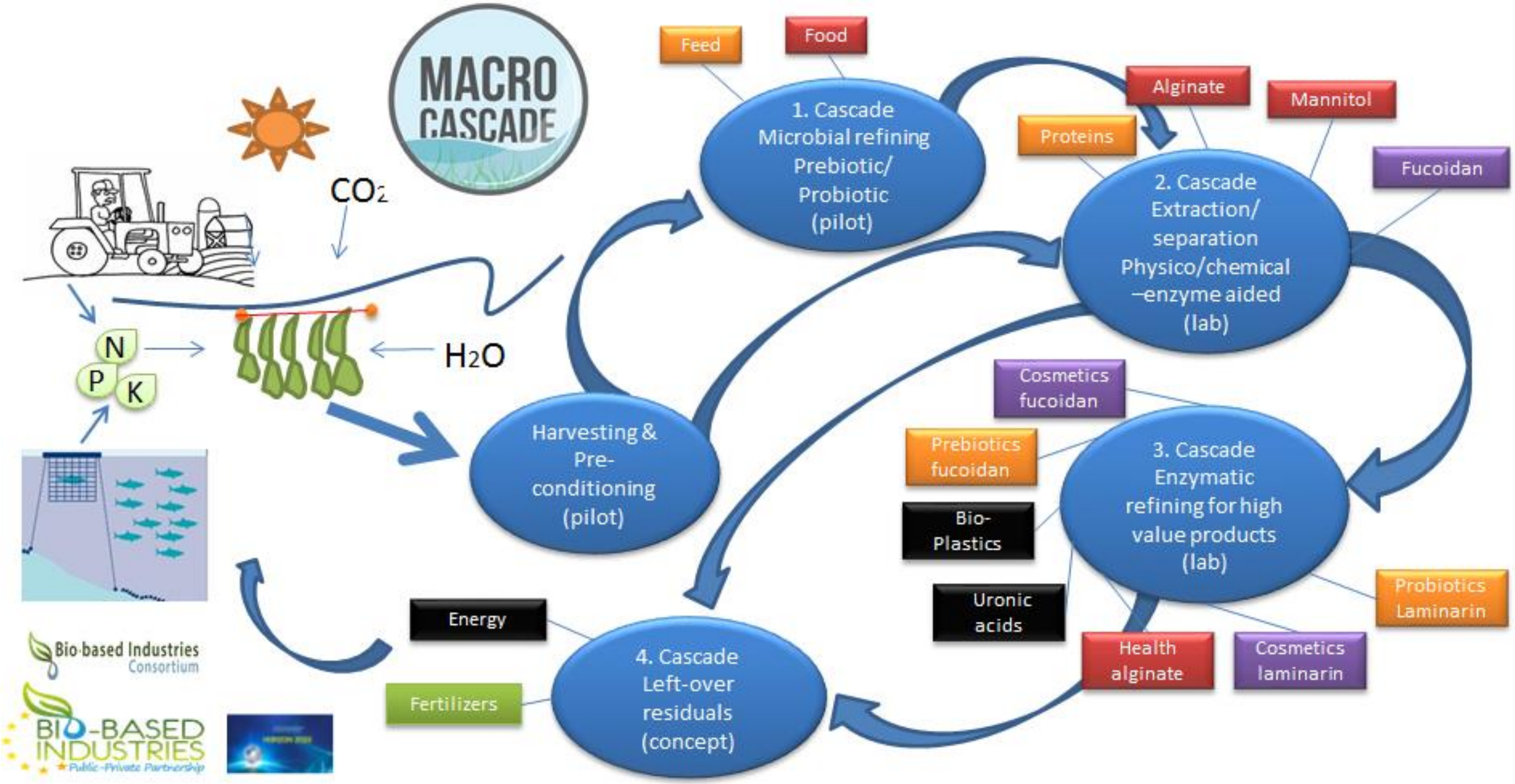
→ **Legarth** next presentation

4) Develop bioactive **food** ingredients with dietary fibers/prebiotics from algae → **Villadsen** next presentation

5) Develop aerobic bioconversion process for production of *prebiotic carbohydrates* and *bio-colorants,* **feed and food**

6) WP5: D5.3. Development of catalytic processes for conversion of fucoidan to bioactives

Macrocascade Concept



MICROBIAL *AND ENZYMATIC* REFINING OF ALGAL BIOMASS FOR FOOD AND FEED INGREDIENTS (WP3)

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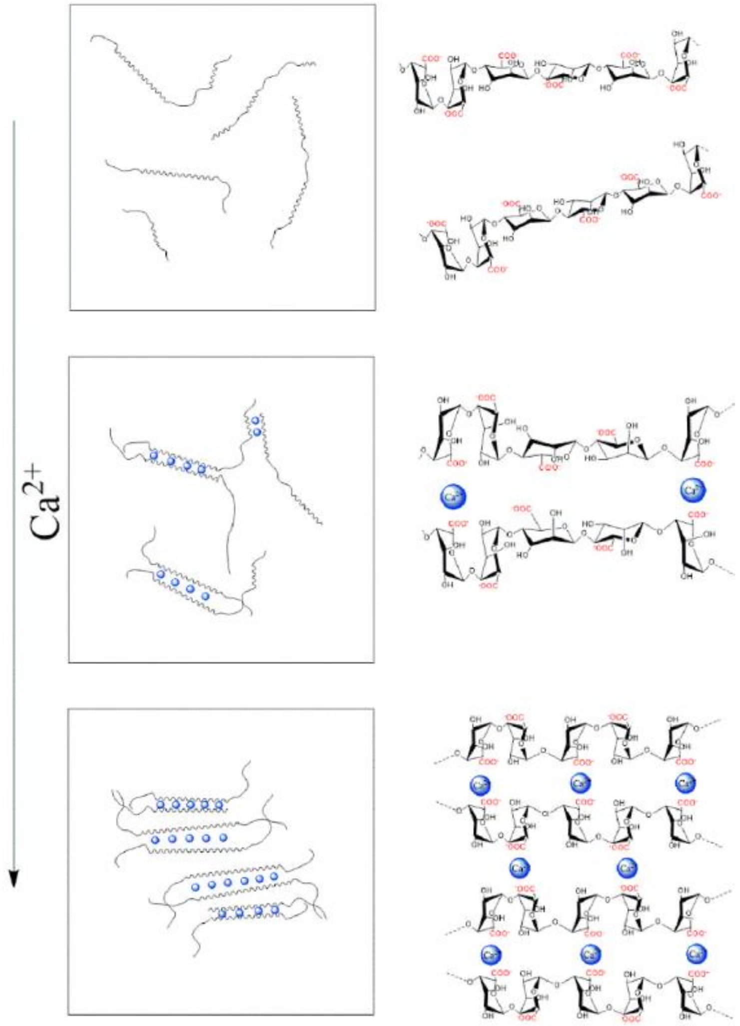
Brown Seaweed Carbohydrate Components

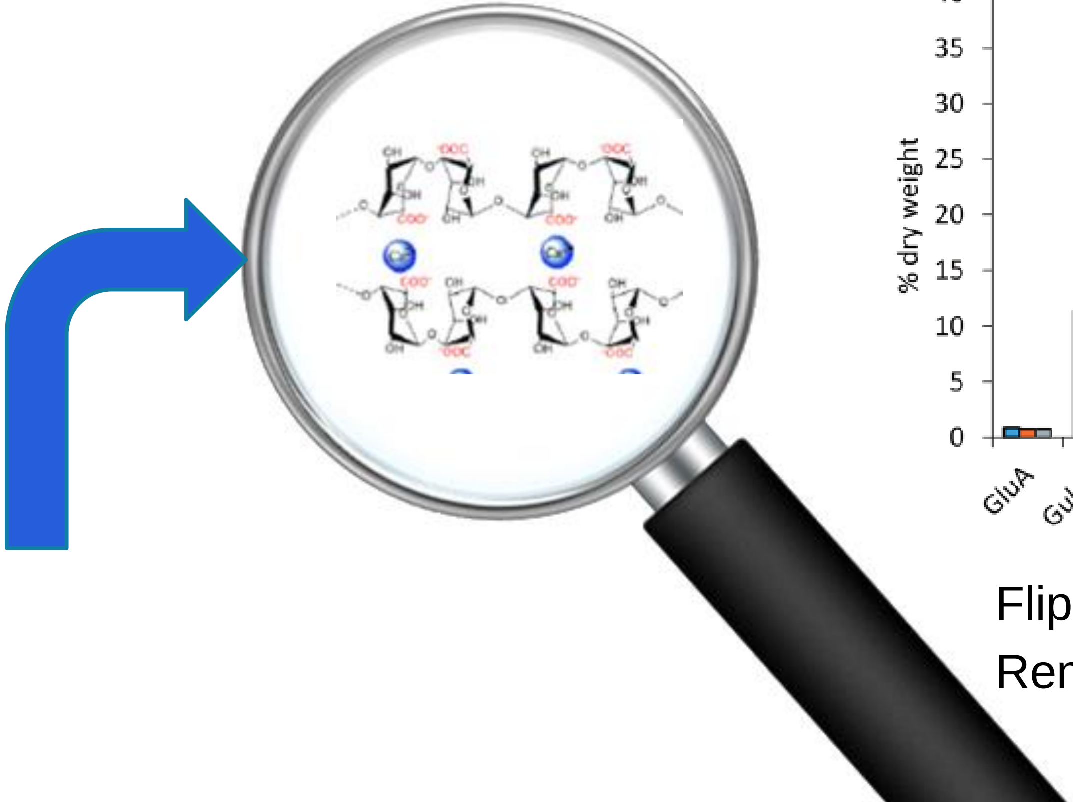
- **Alginate: Hydrocolloid of uronic acids: Gel formation; mostly ‘G’**
- **Laminarin: Potentially bioactive glucan: β-1,3 and β-1,6-linkages**
- **Carbon for microbial carotenoid production & probiotic growth**
- **Fucoidan: Fucose-rich sulfated polysaccharides**
  - **Many high-end biomedical bioactivities**
- **Unique Red Seaweed: *Palmaria palmata;***
  - **mixed-linkage-xylan oligos support growth of *Lactobacillus pentosus***

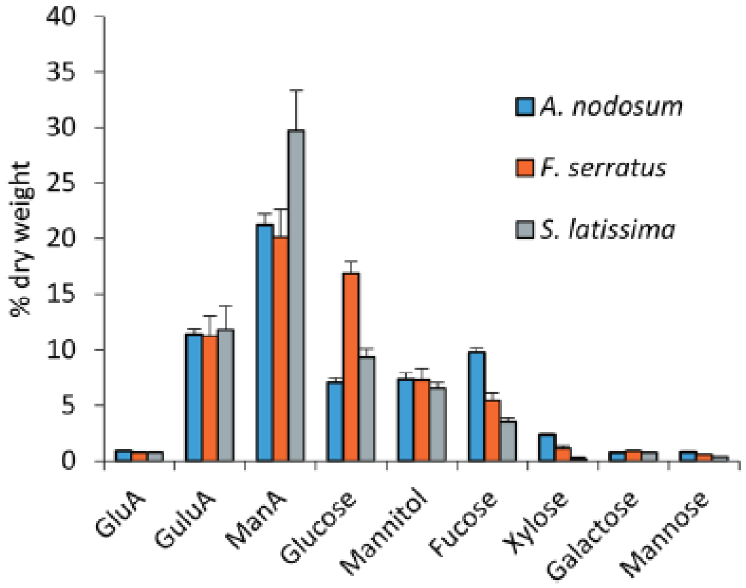
Enzymatic alginate improvement



Gelation: mainly with GGGG-blocks; Make more G from GGMM

Saccharina latissima has high alginate, but very high M:G ratio

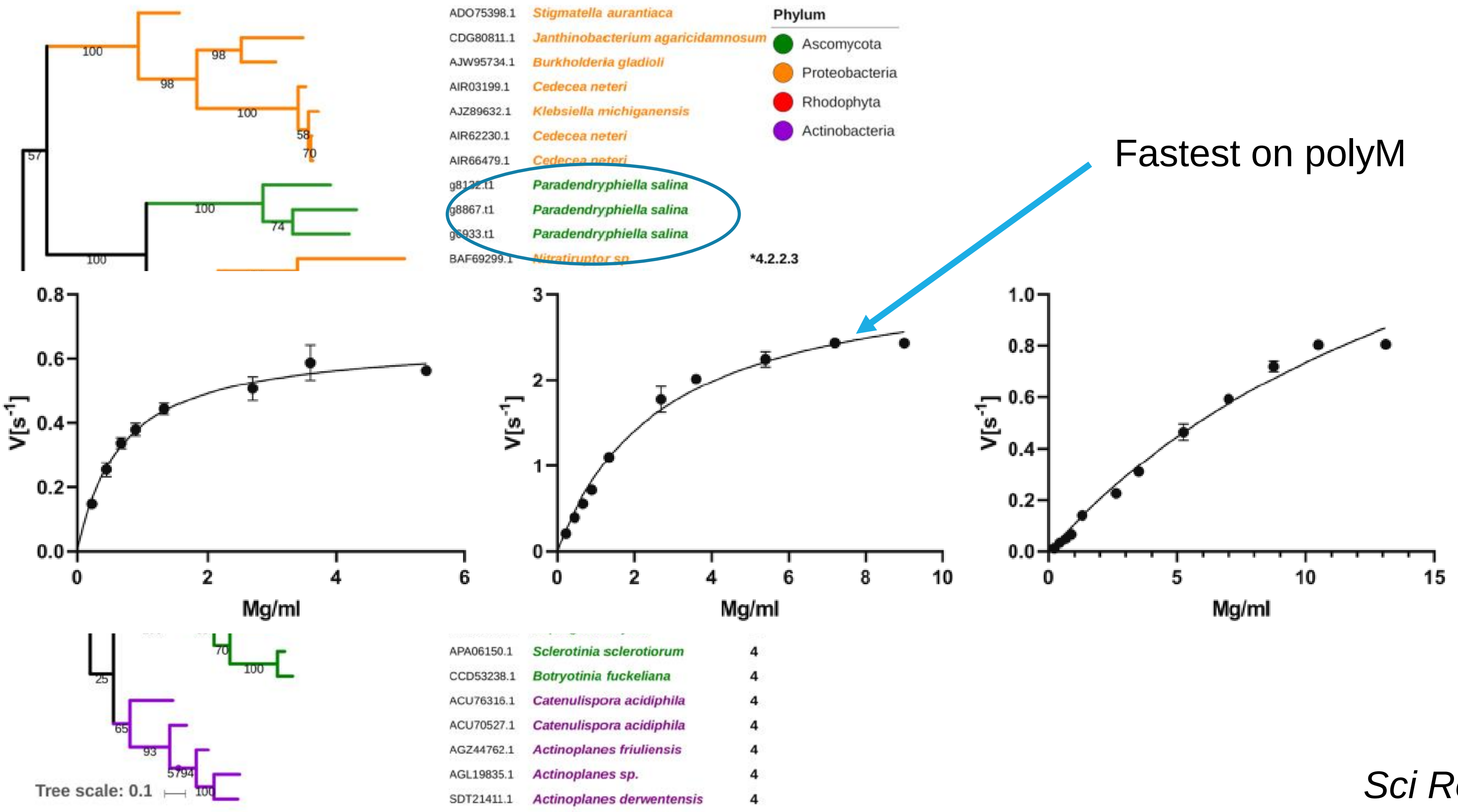




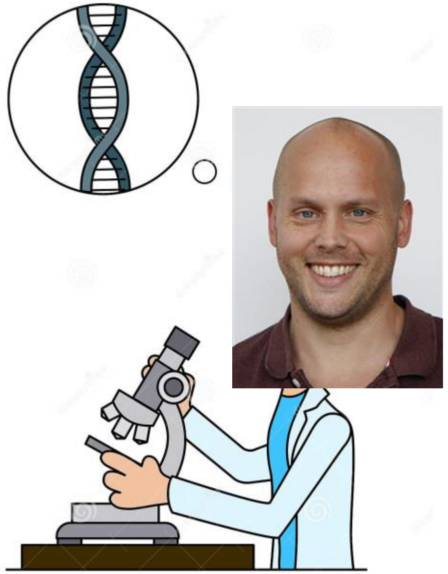
Flip or remove some M

Remove M from chain ends?

Enzymatic alginate improvement



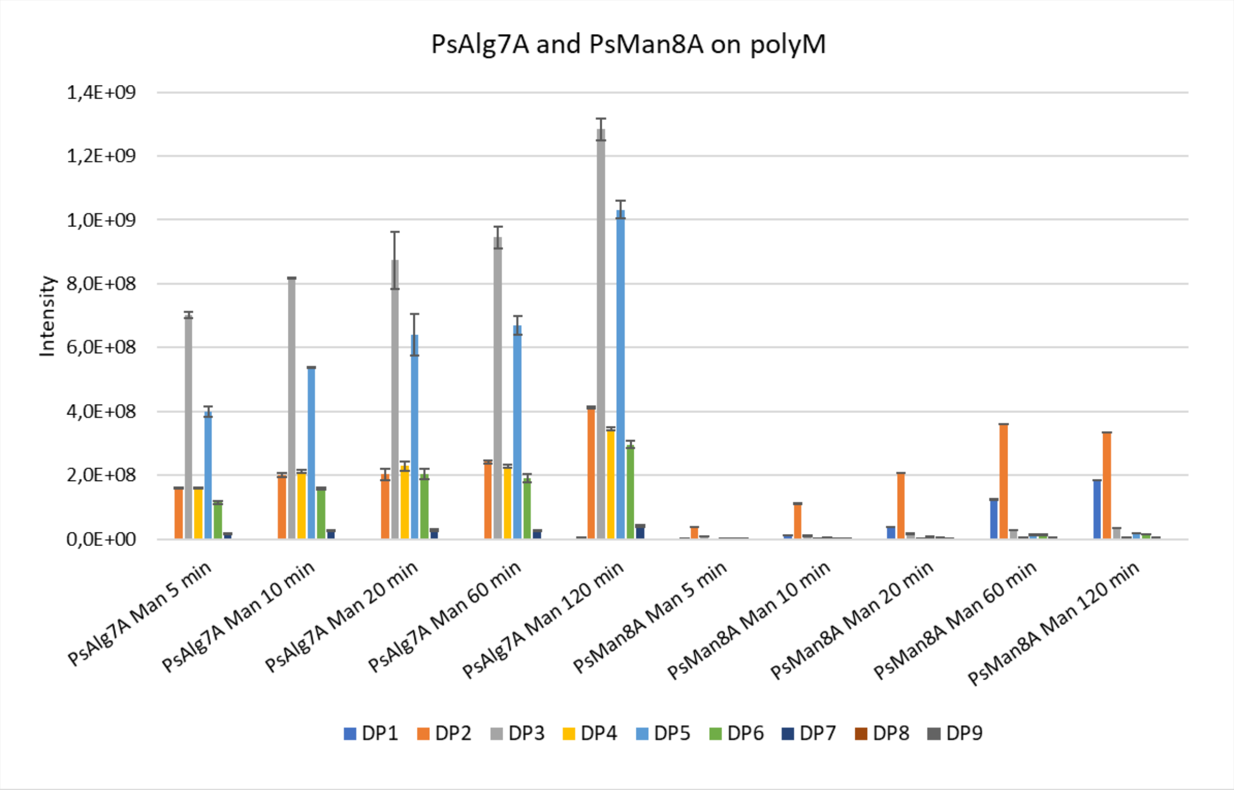
Look for alginate-modifying enzymes

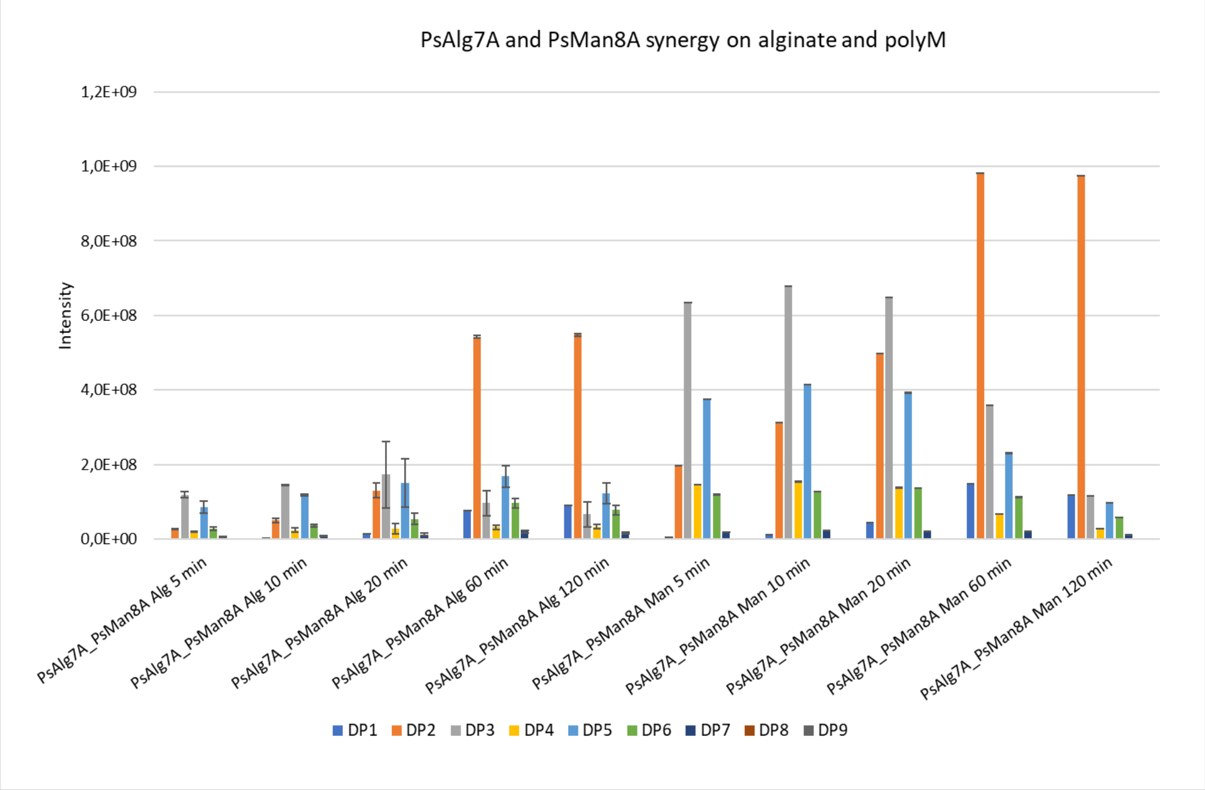


Bo Pilgaard, PhD stud.

Sci Rep 9, 12338-12351, 2019

Enzymatic alginate improvement





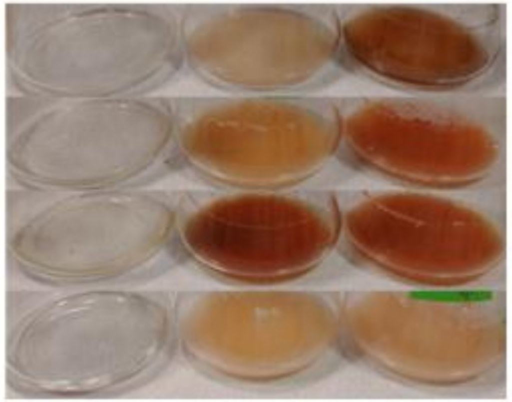
J of Fungi 7, 80-95, 2021

Rhodothermus marinus grows on alginate

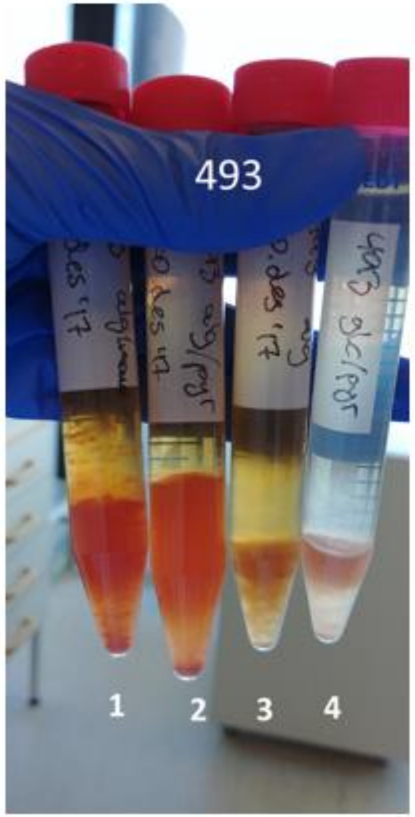
R. marinus grown on alginate

	OD@620nm
376 glc/pyr	5,17
493 glc/pyr	4,09
376 alg	0,87
493 alg	1,17
376 alg/pyr	2,76
493 alg/pyr	2,74
376 alg/mono	3,01
493 alg/mono	3,03
Neg alg	0,093
Neg alg/pyr	0,092
Neg alg/mono	0,071

1.
2.
3.
4.

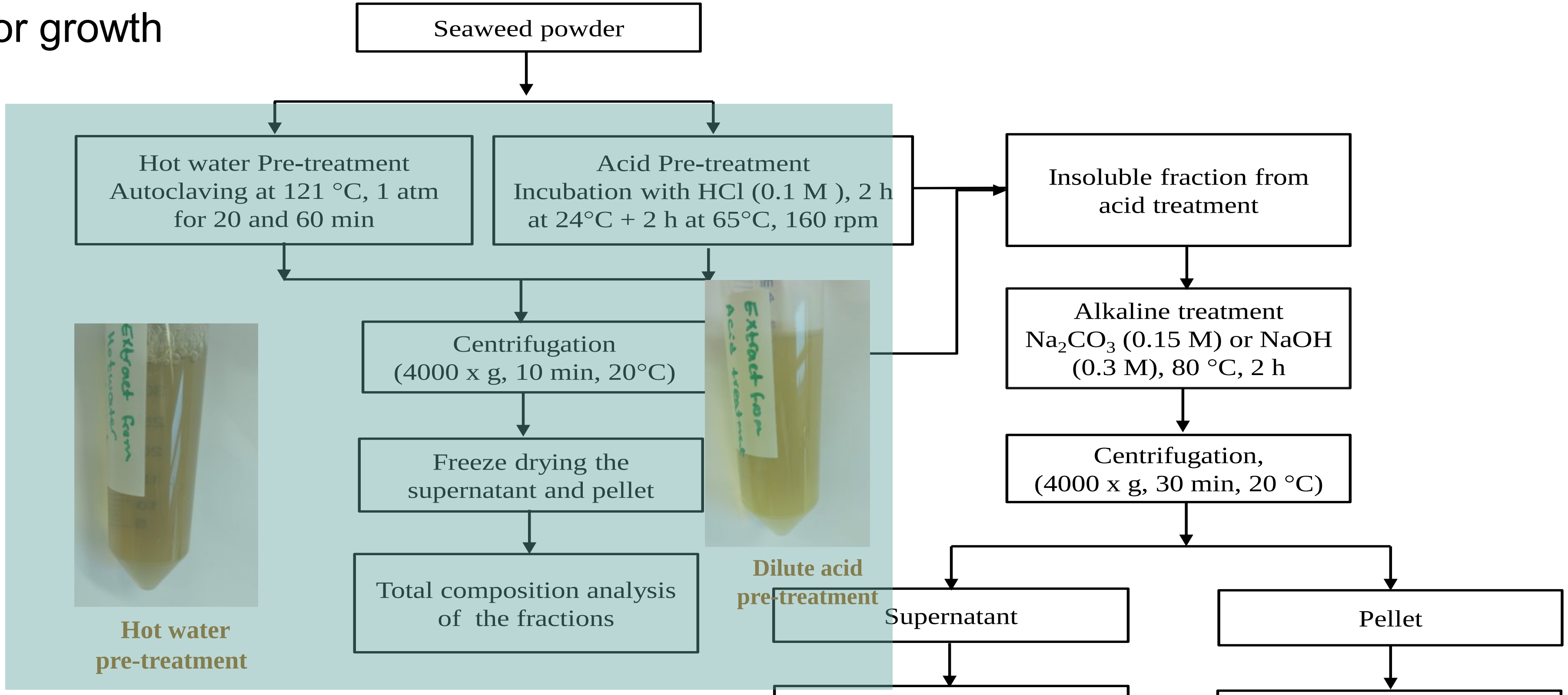


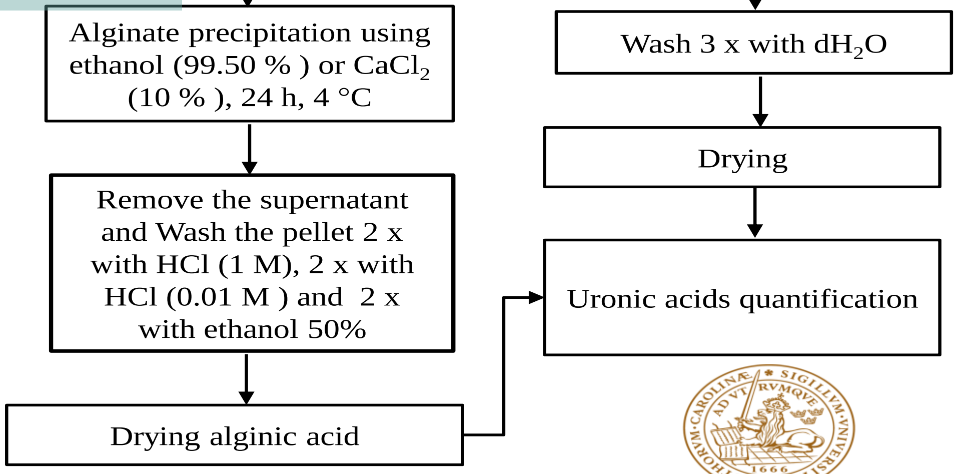
Neg 376 493



- Media tested:
  1. Alginate (1%)
  2. Alginate (1%) + pyruvate (10mM)
  3. Alginate (1%) + monouronic acids
  4. Glucose (10g/l) + pyruvate (10mM)
- Strains; 376, 493 and negative control for all media.
- Conclusion: *R. marinus* can use alginate as the main carbon source producing large quantities of carotenes

"Minimal seweed processing" for growth trials of *R. marinus*





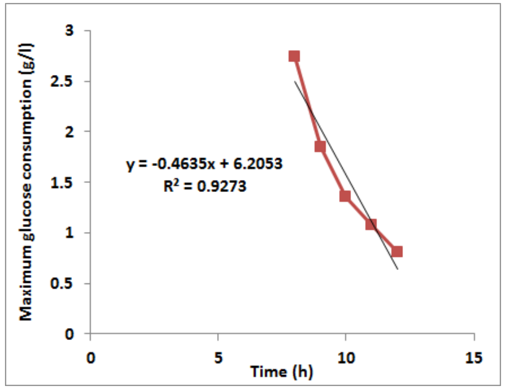
Pretreatment method	Composition				
	Ash (%)	Protein Content (%)	Reducing sugars (mg/g DW[1])	Total Phenolic content (mg GAE/g DW[1])	Total carbohydrate (%)
Hot water (20 min)	48.95±1.87	6.88	12.35 ±0.994	2.84 ± 0.18	34.83 ± 1.52
Hot water (60 min)	45.72±2.76	6.98	17.63 ± 0.714	3.20 ± 0.03	33.53 ± 2.38
HCl (0.1 M)	54.13±0.19	5.26	11.63 ± 0.60	2.83 ± 0.15	25.79 ± 0.4

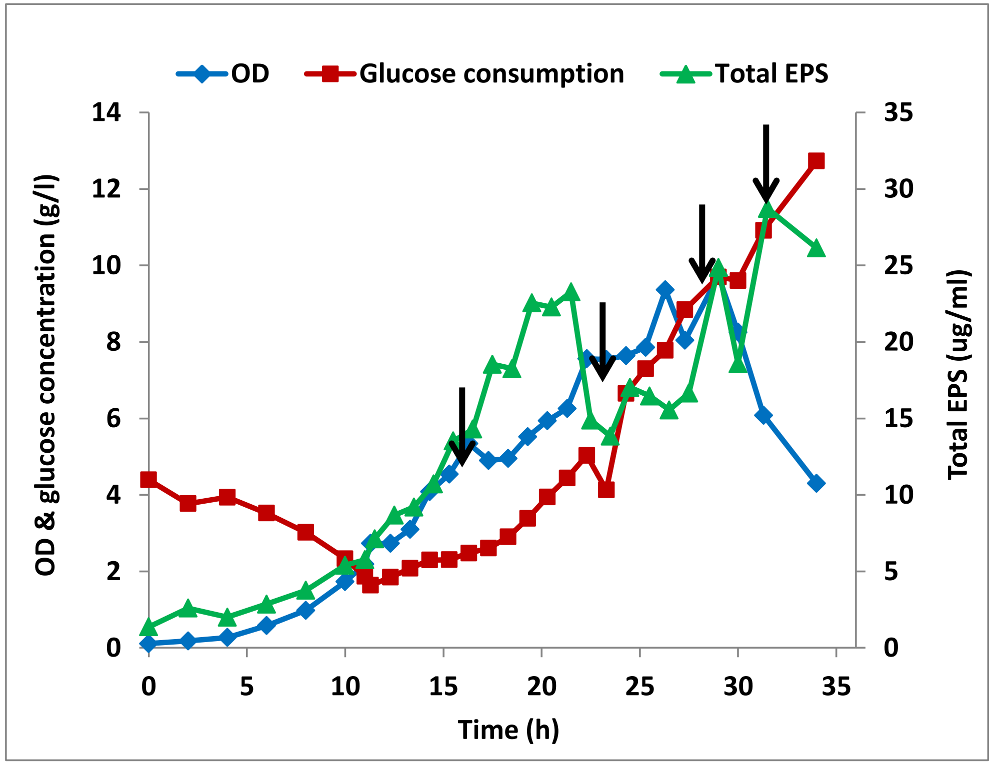
[1]DW means dry weight of the extracts

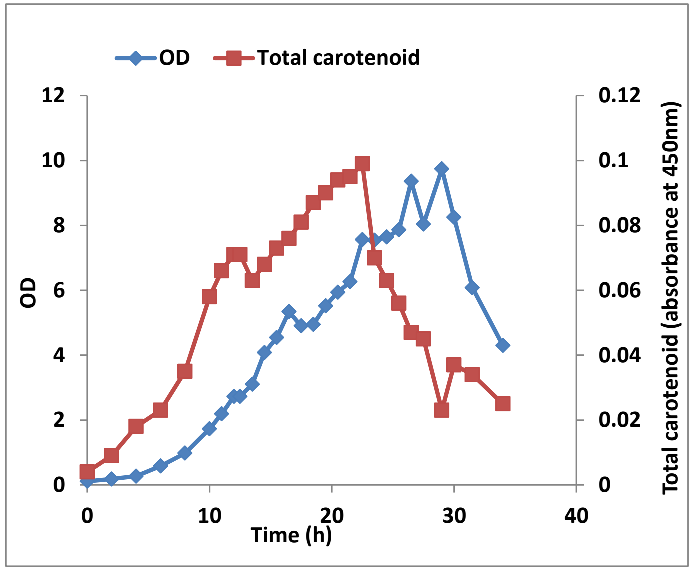
LUND UNIVERSITY

R. marinus Fed-batch cultivation and carotenoid production

- Glucose consumption rate :0.46 g/l.h
- Feed solution: trace elements 100ml/L, glucose 0.56 M (100 g/L), NH_4Cl 0.2 M (10.7 g/L), $CaSO_4$ 2.3 mM and phosphate buffer 200 mM
- Initial working volume of medium in bioreactor: 500ml

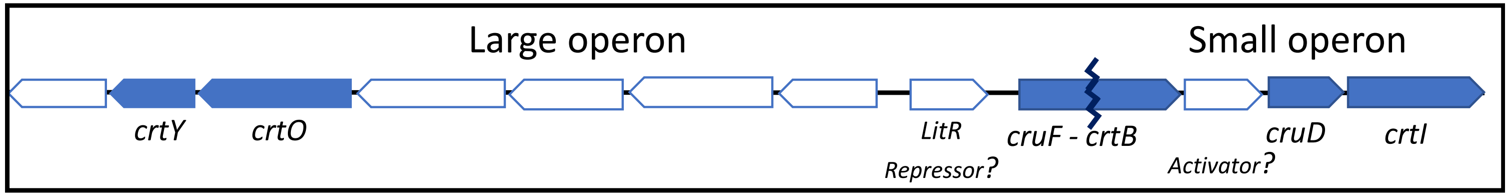






Carotenoids from *Rhodothermus marinus*

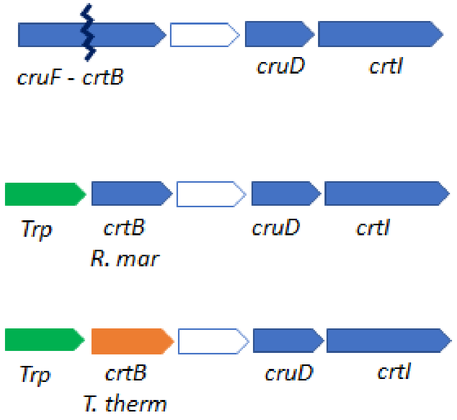
- **Carotenoid identification and engineering**
  - Native carotenoid: a monocyclic γ-carotene (or β,ψ-carotene)
  - Pathway engineering
  - Extraction from *Rhodothermus marinus* strains, and MS identification.
  - Lycopene production in engineered variant confirmed.

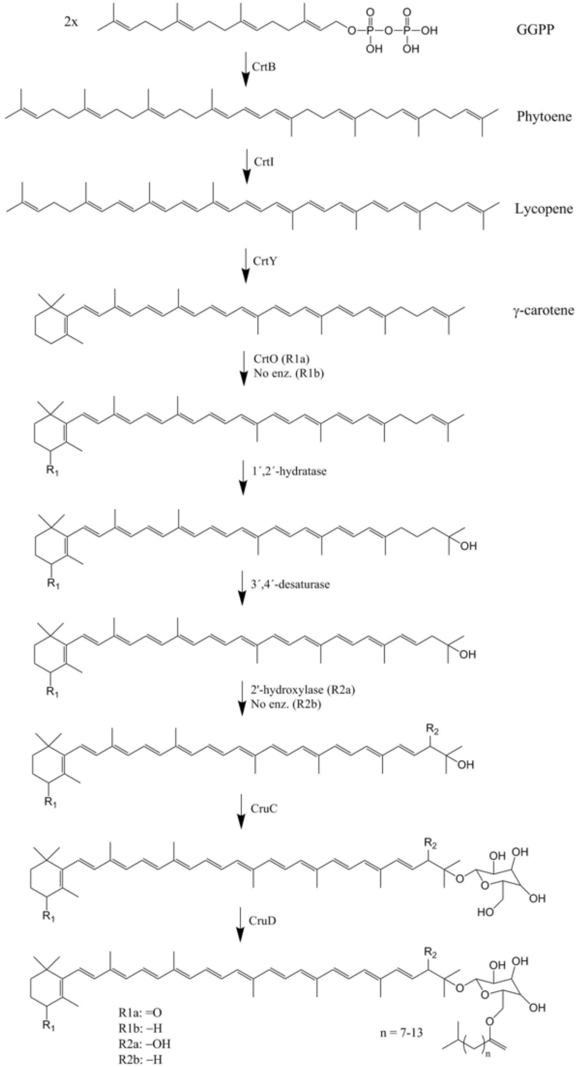


1 Wild-type

2 Only CruF knocked out

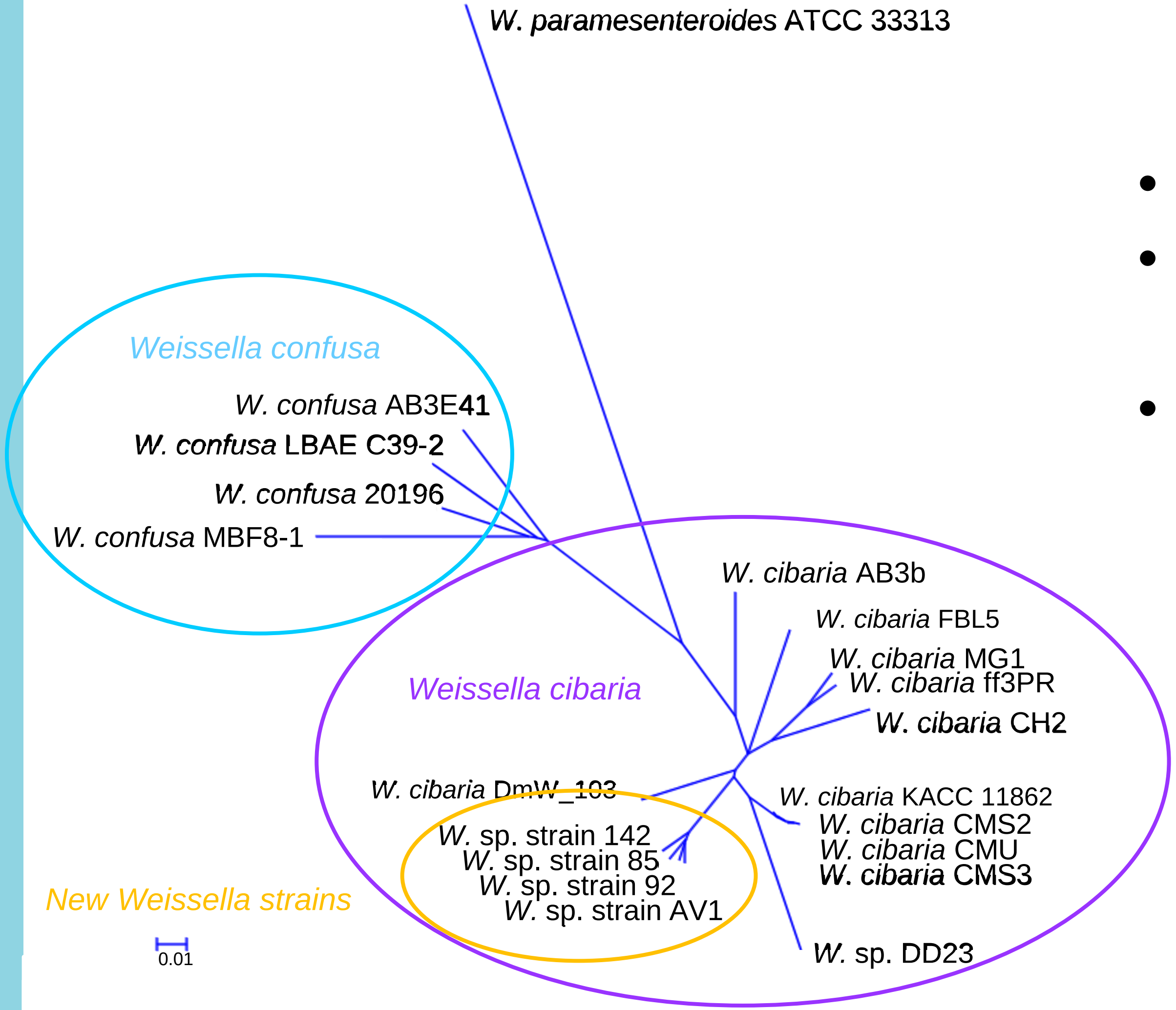
3 CruF-CrtB knocked out
CrtB from *Thermus* inserted





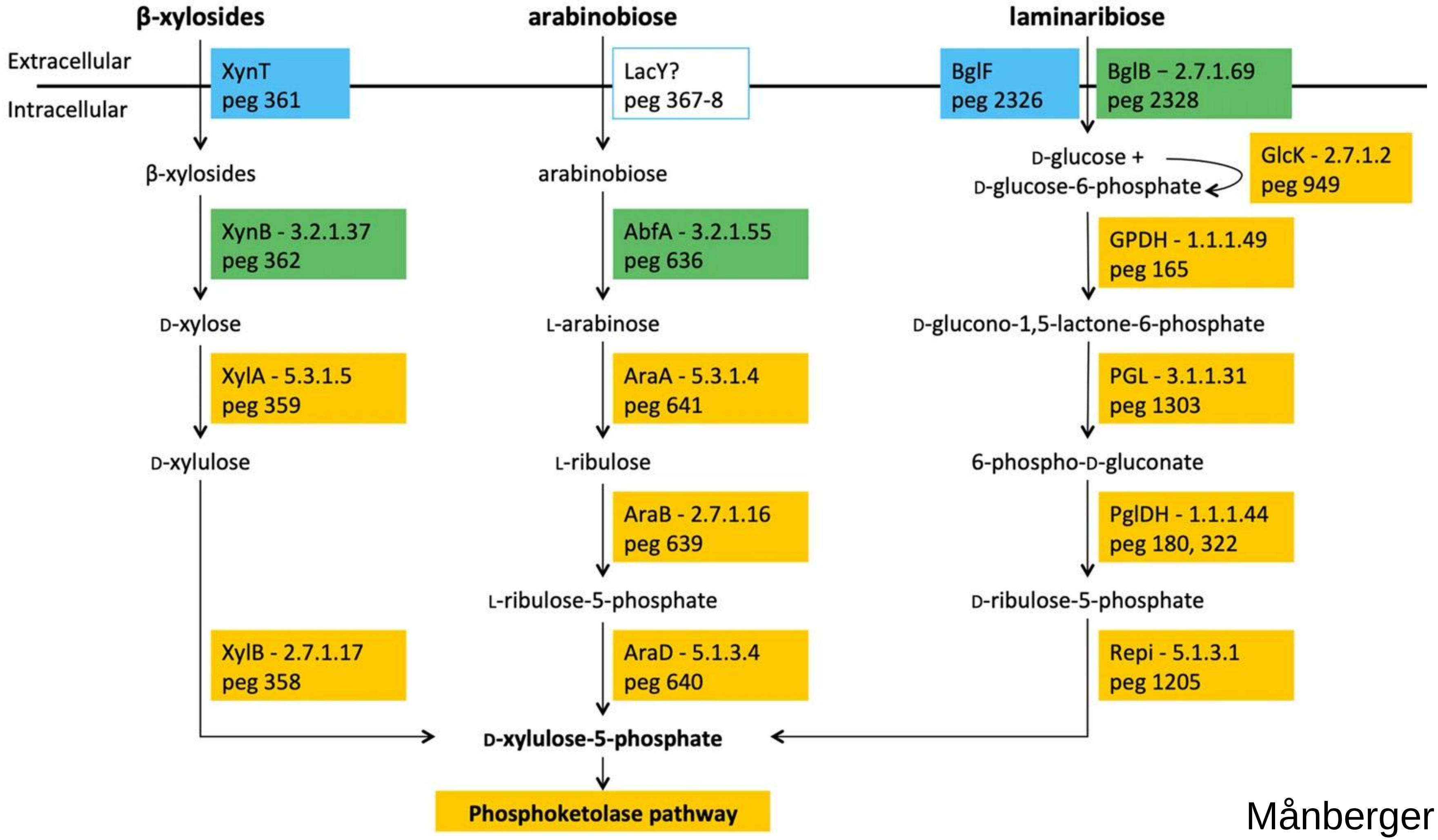
Ron et al, Microbiol open, 2018
Kristjansdottir et al, Met Eng Comm, 2020

New strains of *Weissella cibaria*



- Isolated from Indian fermented food
- *Species identification* based on whole-genome phylogeny
- Potential probiotic

Månberger et al, 2020, Scientific Reports



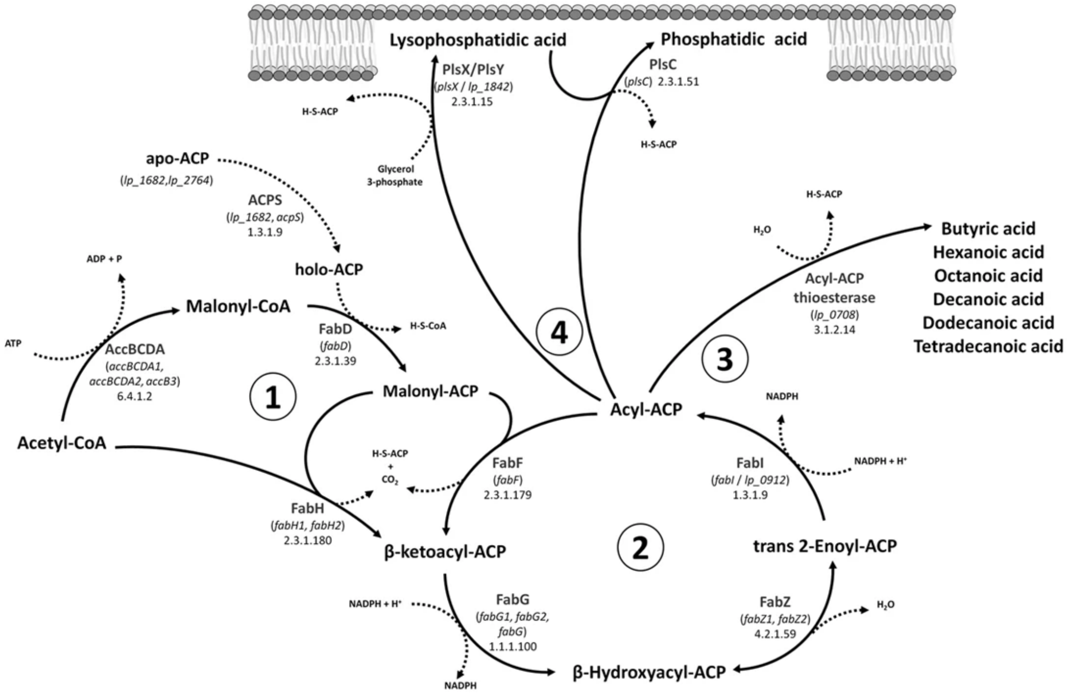
Efficient metabolism of disaccharides, including laminaribiose, arabinobiose and XOS (DP2-3)

Månberger et al, 2020 Scientific Reports

Product profile

Butyrate production occurs via fatty acid synthesis (II) pathway,

(Figure from Botta et al, 2017, Sci. Rep.)



P - production, U - uptake, n.d. – not detected.

Carbohydrate	Carbohydrate fermentation	Fermentation products: Lactate	Ethanol	Acetate	Propionate	Butyrate
1 mL cultivation – microaerophilic						
Glucose	+	P	P	U	n.d.	n.d.
Laminaribiose	+	P	P	P	n.d.	n.d.
Laminaritriose	-					
Laminaritetraose	-					
Arabinose	+	P	n.d.	P	n.d.	n.d.
Arabinobiose (A_2)	+	P	n.d.	P	n.d.	P
Arabinotriose (A_3)	-					
Arabinotetraose (A_4)	-					
Arabinopentose (A_5)	-					
N-Acetyl-glucosamine	+	P	P	P	n.d.	n.d.
Diacetyl-chitobiose	-					
75 mL cultivation – anaerobic						
Glucose	+	P	P	P	n.d.	n.d.
Arabinose	+	P	n.d.	P	n.d.	P
Xylose	+	P	n.d.	P	n.d.	P
Negative control	-					

Månberger et al, 2020, Sci Reports

Fucoidans

Maria Dalgaard Mikkelsen

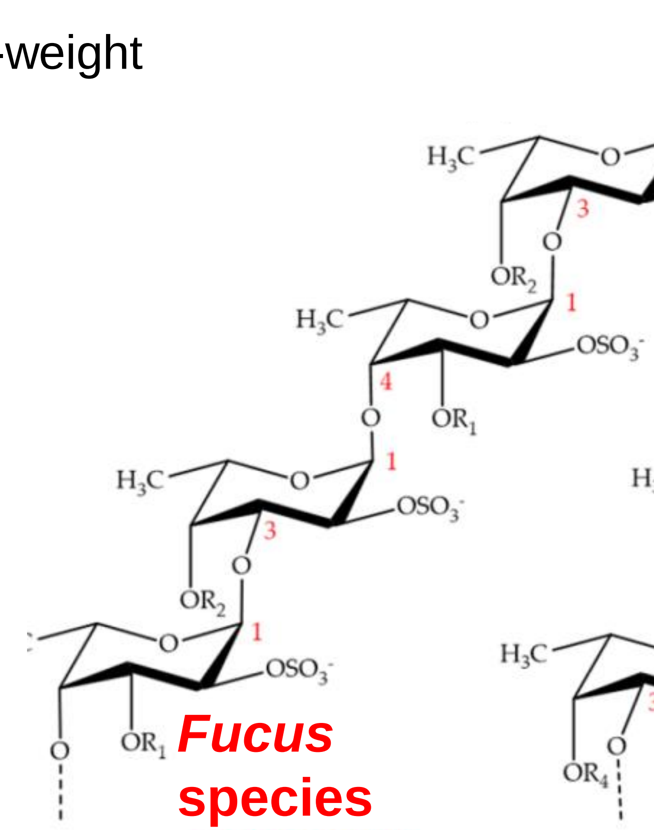
Fucoidans:

2-10% of brown seaweed dry-weight

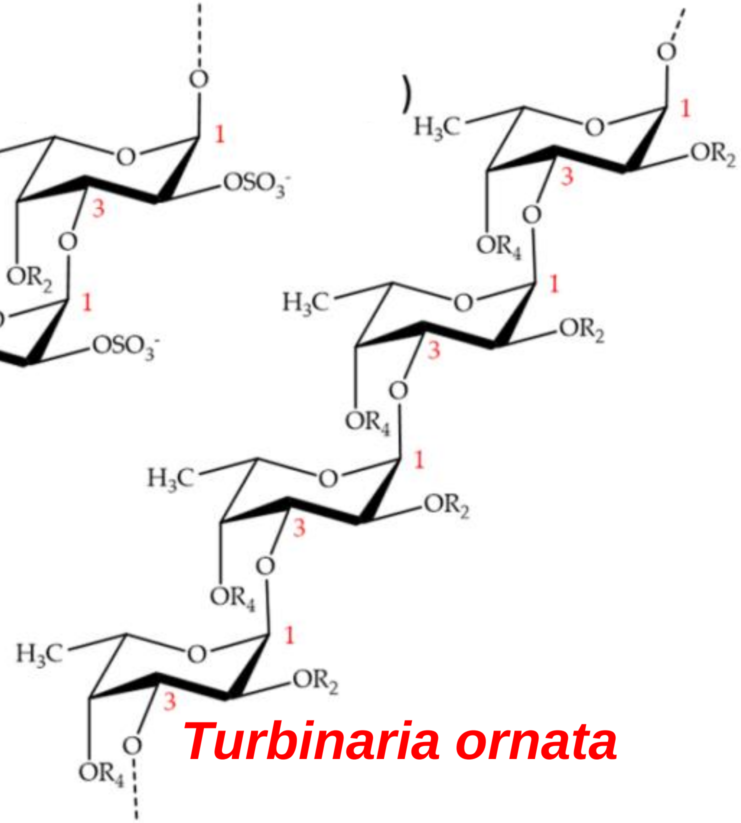
Complex structure and high molecular weight

Bioactive compound

- Anti-inflammatory
- Anti-oxidant
- Anti-tumoral
- Anti-viral
- Anti-coagulant
- Anti-thrombotic
- Immunomodulatoric

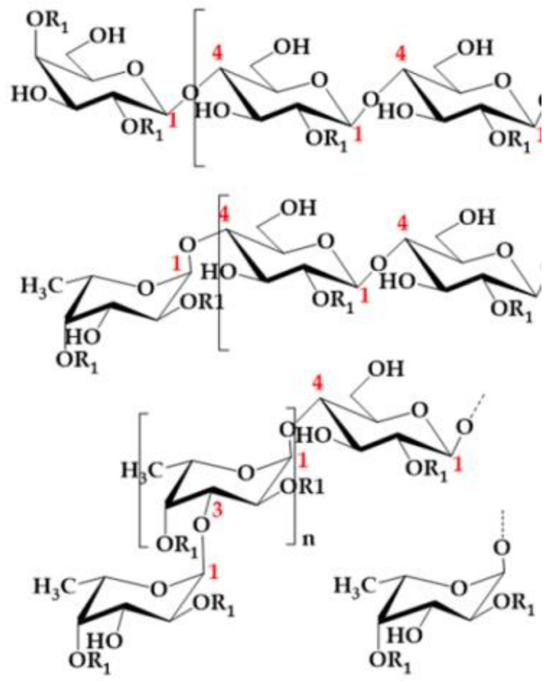


Fucus species



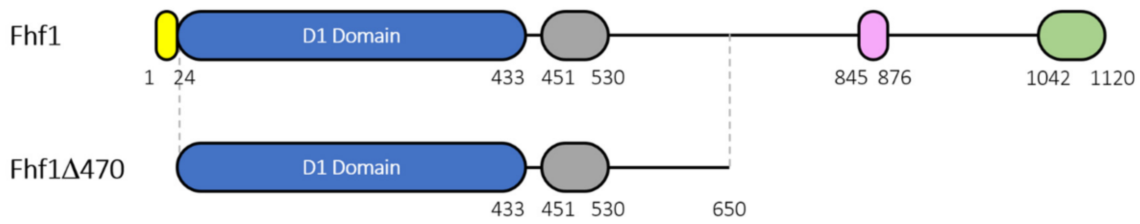
Turbinaria ornata

Side branches

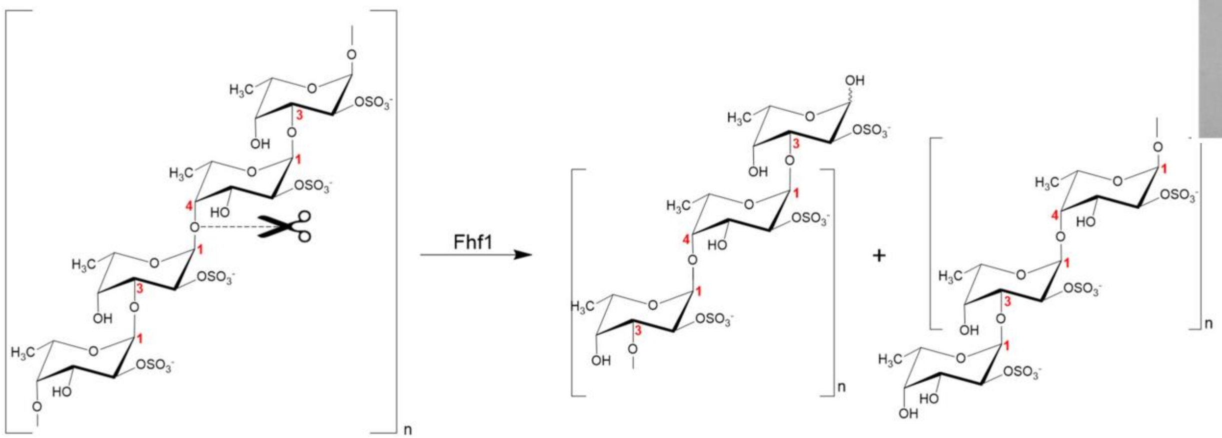


The fucoidanase Fhf1 from *Formosa haliotis*

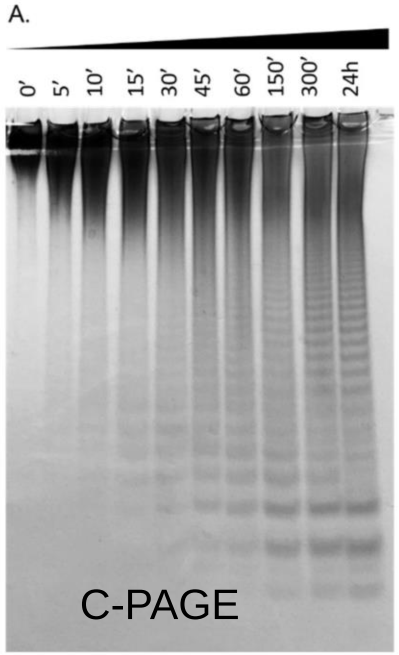
Construction of C-terminally truncated mutant, to obtain good expression

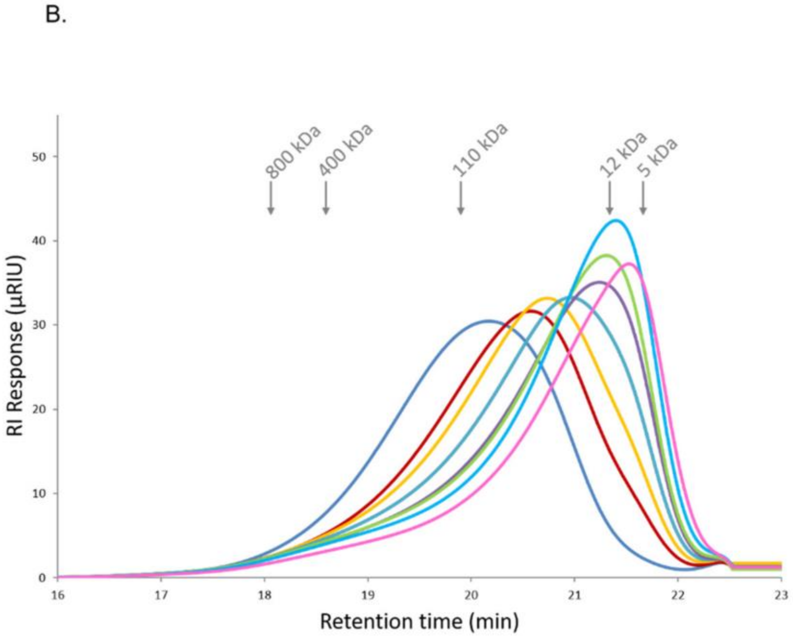


Endo-α-(1,4) linkages (C2 sulfated)



Time-course





SEC

marine drugs

MDPI

Article

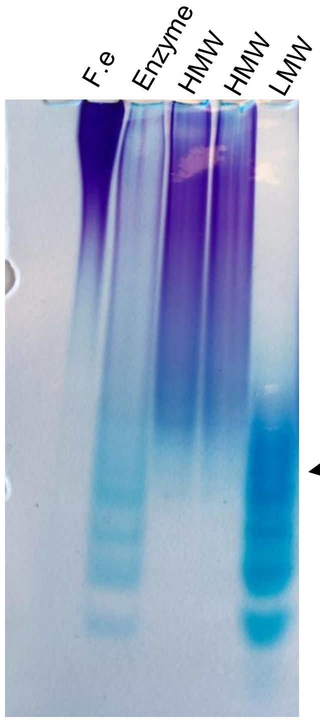
Functional Characterization of a New GH107 Endo-α-(1,4)-Fucoidanase from the Marine Bacterium *Formosa haliotis*

Marlene Vuillemin [1], Artem S. Silchenko [3], Hang Thi Thuy Cao [2], Maxim S. Kokoulin [3], Vo Thi Dieu Trang [1,2], Jesper Holck [1], Svetlana P. Ermakova [3], Anne S. Meyer [1] and Maria Dalgaard Mikkelsen [1,*]

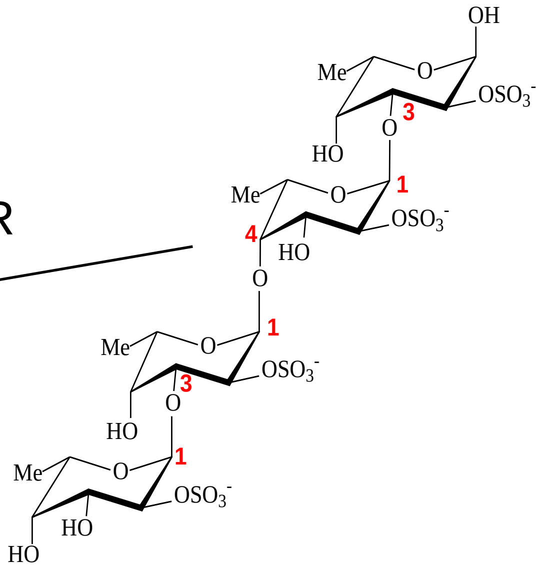
Fhf1 fucoidan oligosaccharides (NMR)

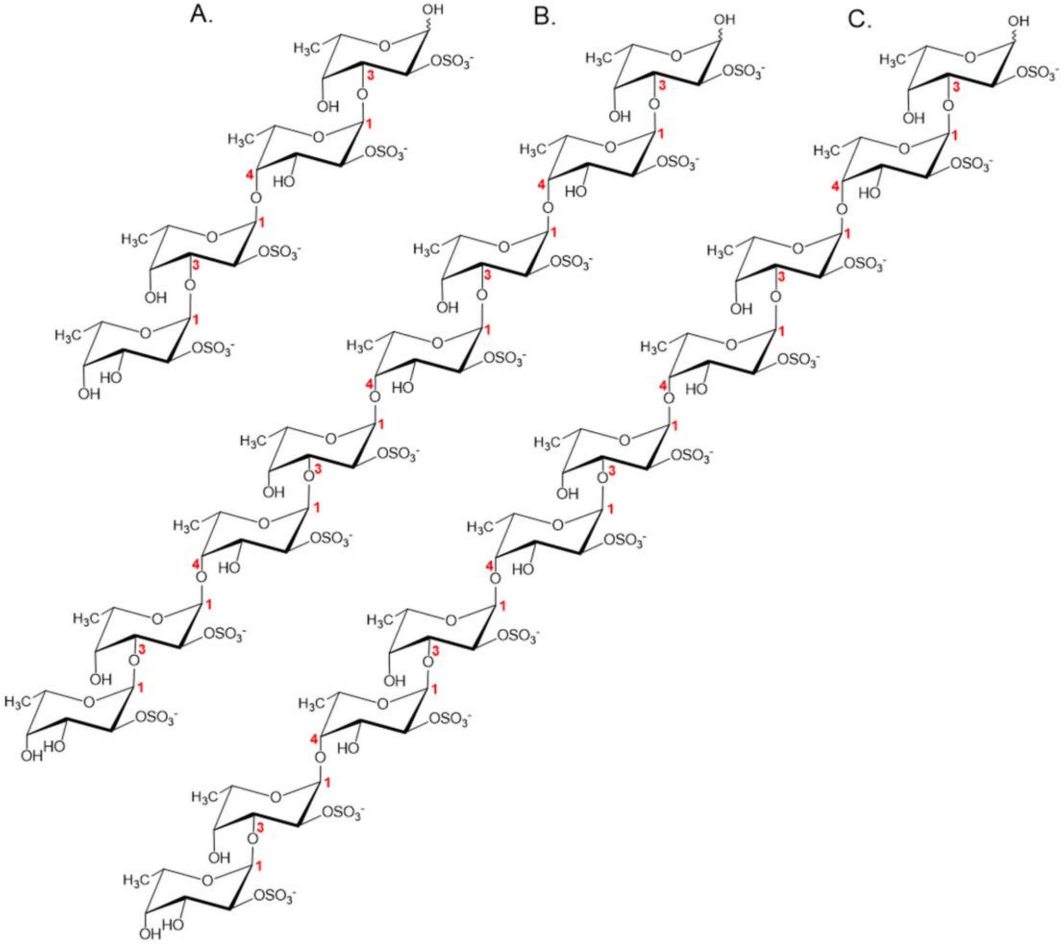
Maria Dalgaard Mikkelsen

C-PAGE



NMR





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