WP5: Enzymatic refining of polysaccharides from Brown seaweed

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WP5: Enzymatic refining for added-value products

Main bulk products from seaweeds are produced using **mechanical and/or physicochemical extraction/fractionation technologies**.

- Seaweed meal
- Hydrocolloids: alginate, carrageenan ..

Biocatalytic" tools", enzymes and microbes, may offer new product possibilities and greater resource efficiency besides being environmental benign options to harsh physicochemical processes harmful to the environment.

- Fermented seaweeds
- Bioactive oligosaccharides,
- Fermentable sugars,
- Rare sugars,
- Platform- and specialty chemicals,
- Energy carriers (biofuels)

Possibilities are largely unexplored,

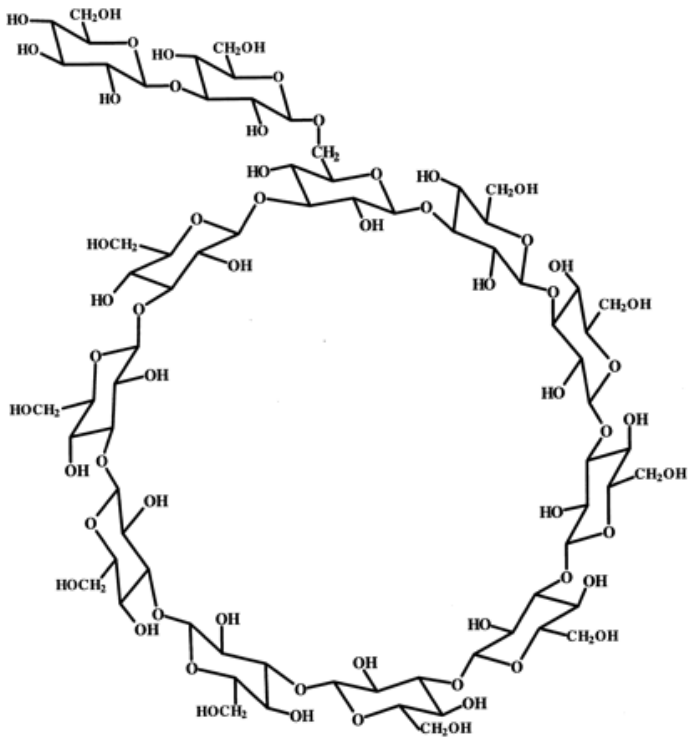
Enzymatic refining of polysaccharides from Brown macroalgae

Laminarin

A 1,3-(1,6)-beta-glucan

Aim: to use enzymes to make branched oligosaccharides enhanced in bioactivity

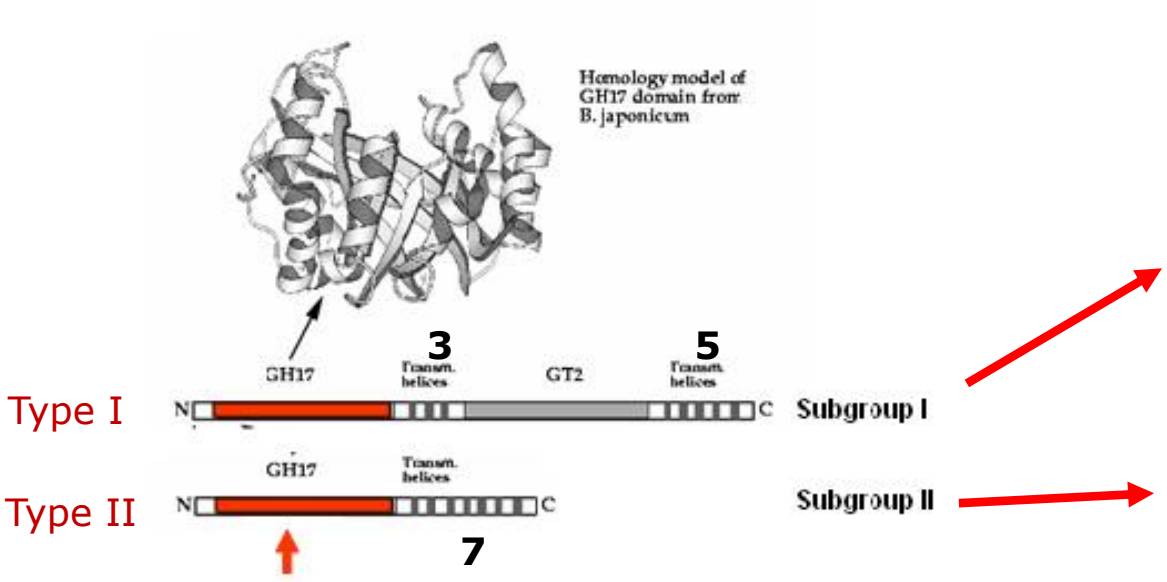
Investigated enzymes that could circularize beta-glucans in *Proeobacteria*

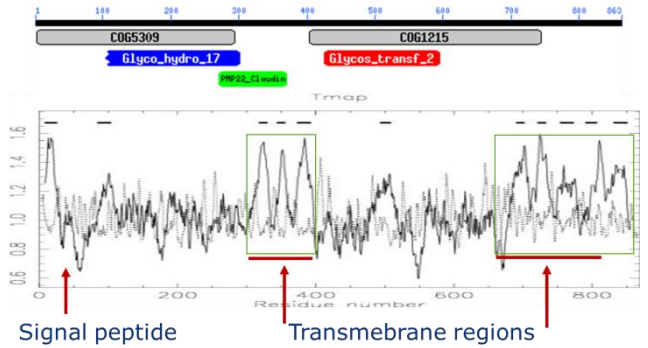


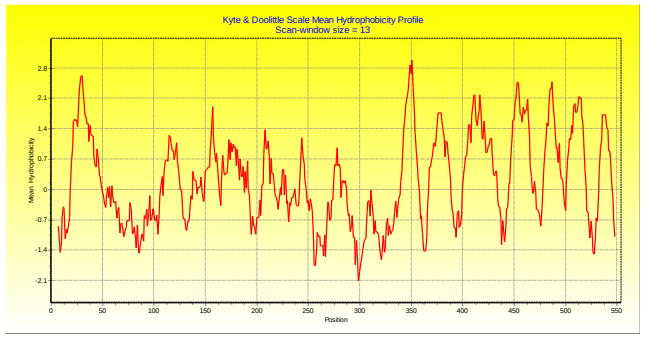
Membrane bound enzymes in Proteobacteria:

Type I: a two domain protein including a GH17 domain and a GT2 domain with two transmembrane regions.

Type II: a GH17 with one region: 7 transmembrane helices at the C terminal end.

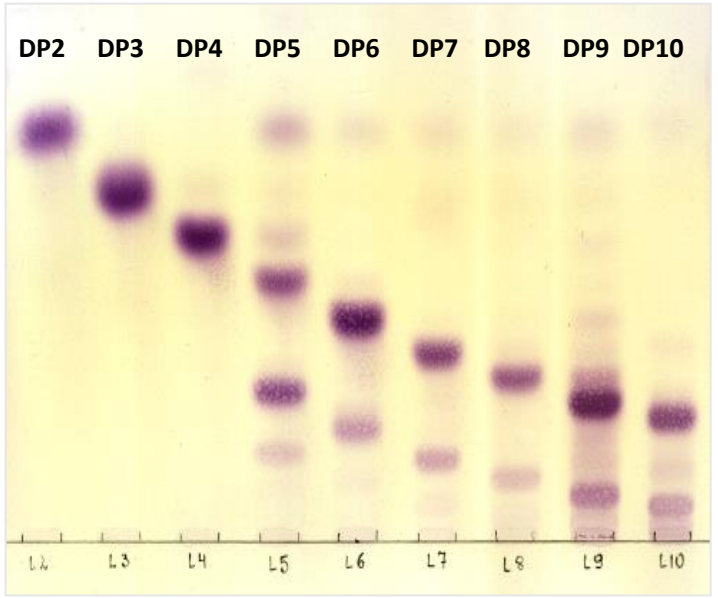






Activity of the Type I **GH17** catalytic domain

TLC analysis



Laminari-oligosaccharides as substrates

Lam-Glc_2

Lam-Glc_3

Lam-Glc_4

Lam-Glc_5

Lam-Glc_6

Lam-Glc_7

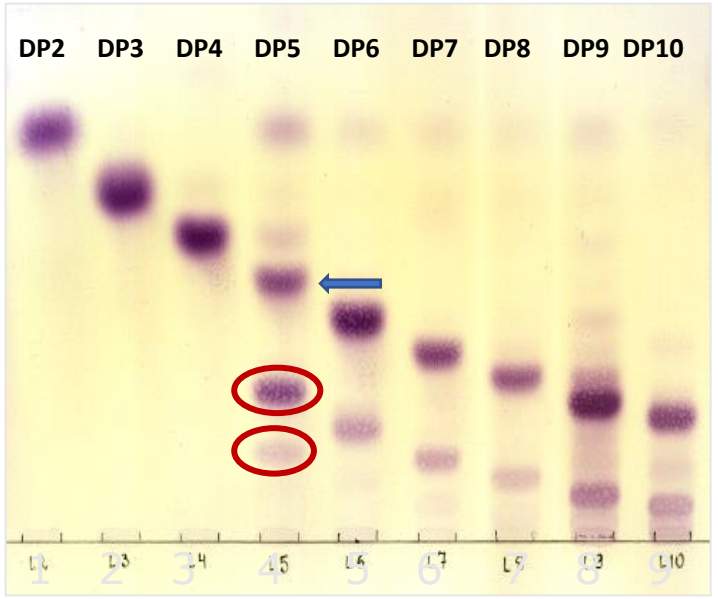
Lam-Glc_8

Lam-Glc_9

Lam-Glc_{10}

Activity of the Type I **GH17** catalytic domain

TLC analysis



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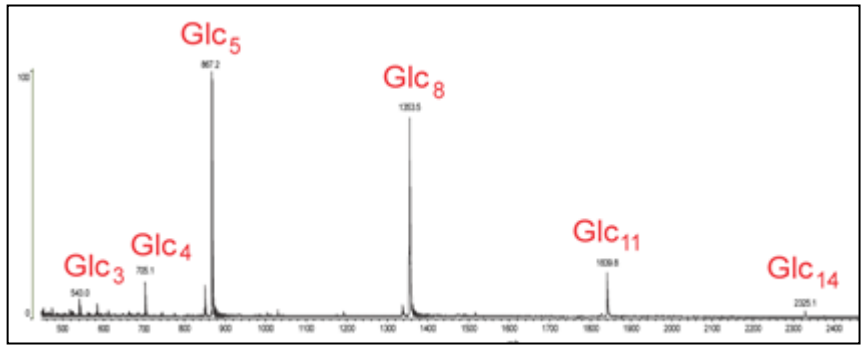
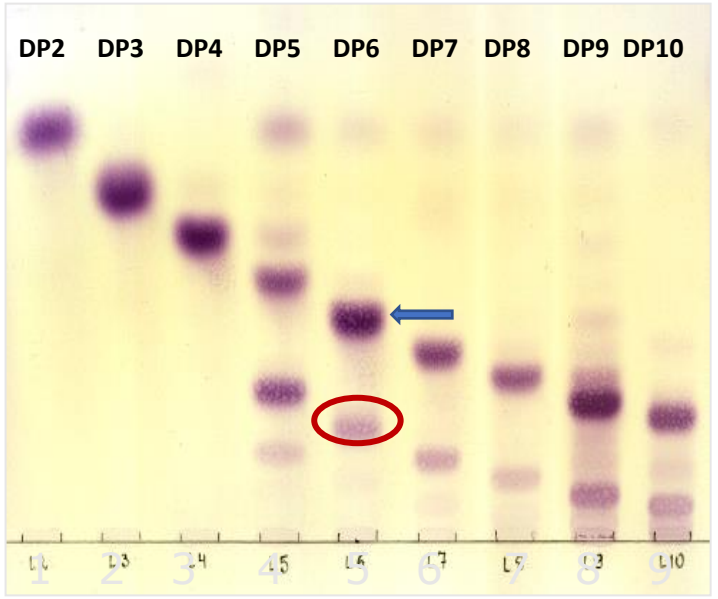
Lam-Glc_{10}



Substrate	Products
DP5	**DP8** (DP3 +DP5) **DP11** (DP3 +DP8)

Activity of the Type I **GH17** catalytic domain

TLC analysis



Substrate	Products
DP6	**DP10** (DP4 +DP6)
	DP14 (DP4 + DP10)

Laminari-oligosaccharides as substrates

Lam-Glc2

Lam-Glc3

Lam-Glc4

Lam-Glc5

Lam-Glc6

Lam-Glc7

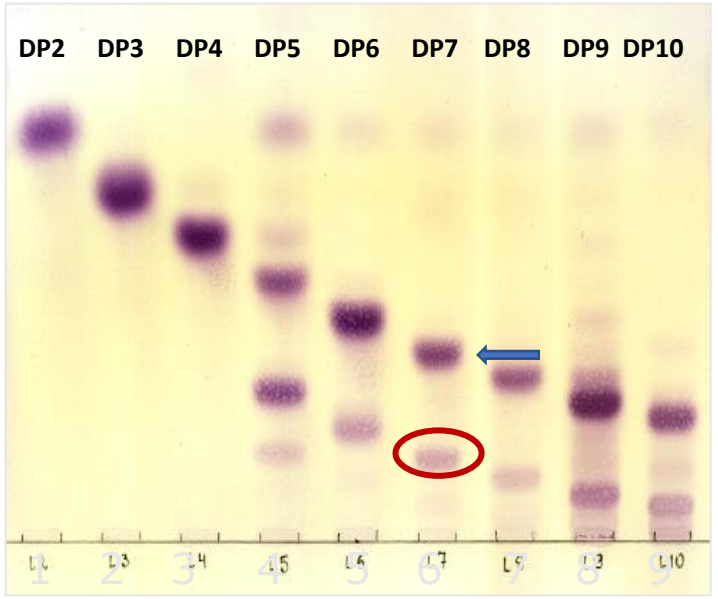
Lam-Glc8

Lam-Glc9

Lam-Glc10

Activity of the Type I **GH17** catalytic domain

TLC analysis



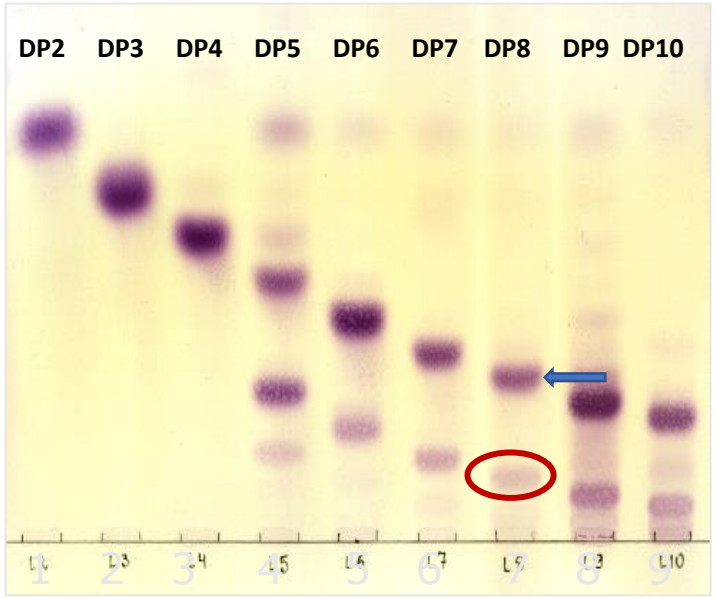
Substrate	Products
DP7	**DP12** (DP5 +DP7)
	DP17 (DP5 +DP12)

Laminari-oligosaccharides as substrates

Lam-Glc2
Lam-Glc3
Lam-Glc4
Lam-Glc5
Lam-Glc6
Lam-Glc7
Lam-Glc8
Lam-Glc9
Lam-Glc10

Activity of the Type I **GH17** catalytic domain

TLC analysis



Substrate	Products
DP8	**DP14** (DP6 +DP8)
	DP20 (DP6 +DP14)

Laminari-oligosaccharides as substrates

Lam-Glc2

Lam-Glc3

Lam-Glc4

Lam-Glc5

Lam-Glc6

Lam-Glc7

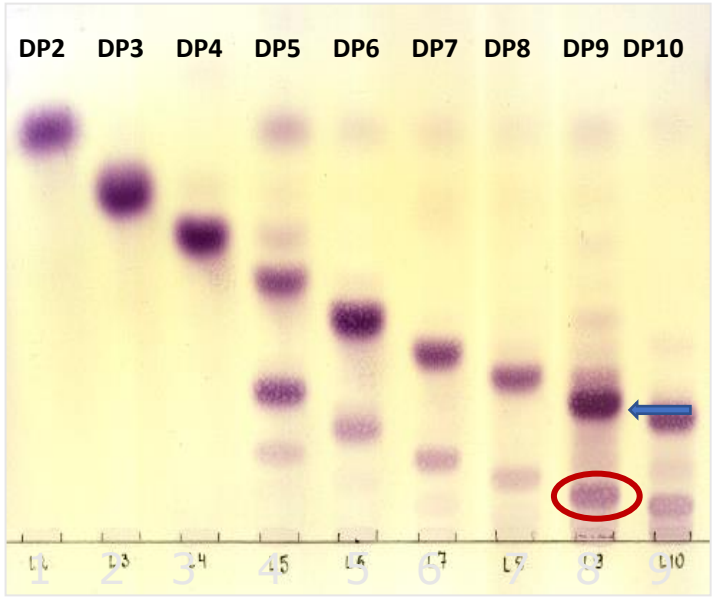
Lam-Glc8

Lam-Glc9

Lam-Glc10

Activity of the Type I **GH17** catalytic domain

TLC analysis



Substrate	Product
DP9	**DP16** (DP7 +DP9)
	DP26 (DP7 +DP16)

Laminari-oligosaccharides as substrates

Lam-Glc2

Lam-Glc3

Lam-Glc4

Lam-Glc5

Lam-Glc6

Lam-Glc7

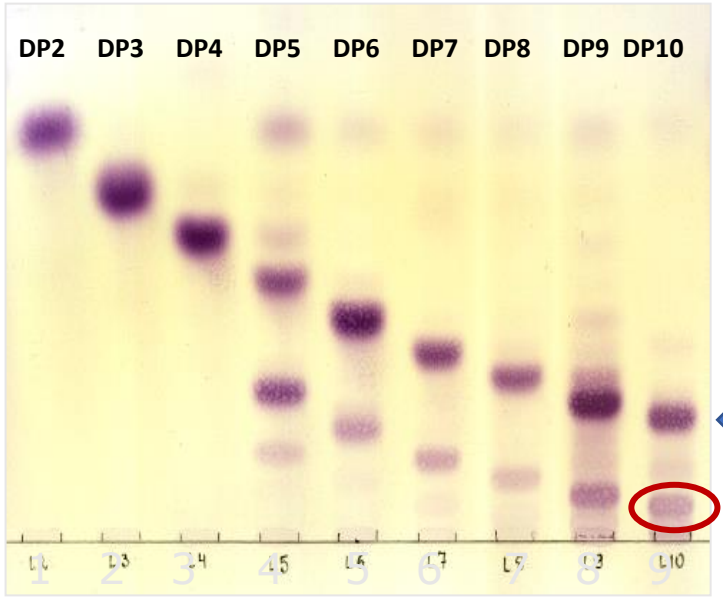
Lam-Glc8

Lam-Glc9

Lam-Glc10

Activity of the Type I **GH17** catalytic domain

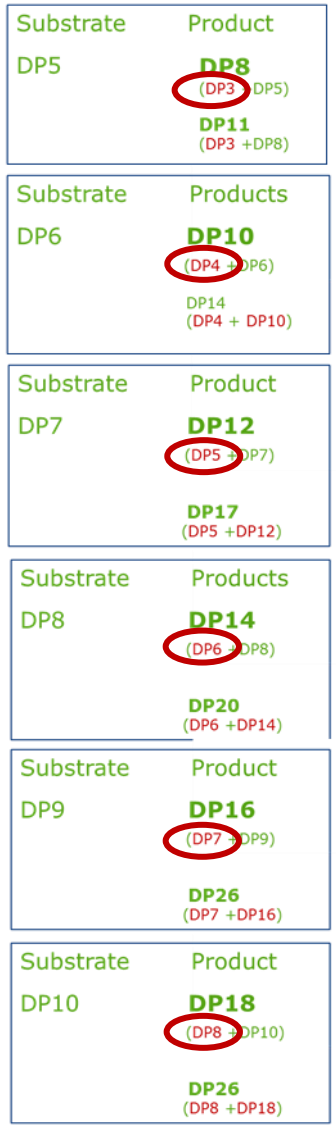
TLC analysis



Laminari-oligosaccharides as substrates

Lam-Glc2
Lam-Glc3
Lam-Glc4
Lam-Glc5
Lam-Glc6
Lam-Glc7
Lam-Glc8
Lam-Glc9
Lam-Glc10

Substrate	**Product**
DP10	**DP18** (DP8 +DP10)
	DP26 (DP8 +DP18)



Comparison of the activity GH17 domains from two different species

Glt20

Substrate	Product
DP5	**DP8** (DP3 + DP5)
	DP11 (DP3 +DP8)

Substrate	Products
DP6	**DP10** (DP4 + DP6)
	DP14 (DP4 + DP10)

Substrate	Product
DP7	**DP12** (DP5 + DP7)
	DP17 (DP5 +DP12)

Substrate	Products
DP8	**DP14** (DP6 + DP8)
	DP20 (DP6 +DP14)

Substrate	Product
DP9	**DP16** (DP7 + DP9)
	DP26 (DP7 +DP16)

Substrate	Product
DP10	**DP18** (DP8 + DP10)
	DP26 (DP8 +DP18)

Glt7

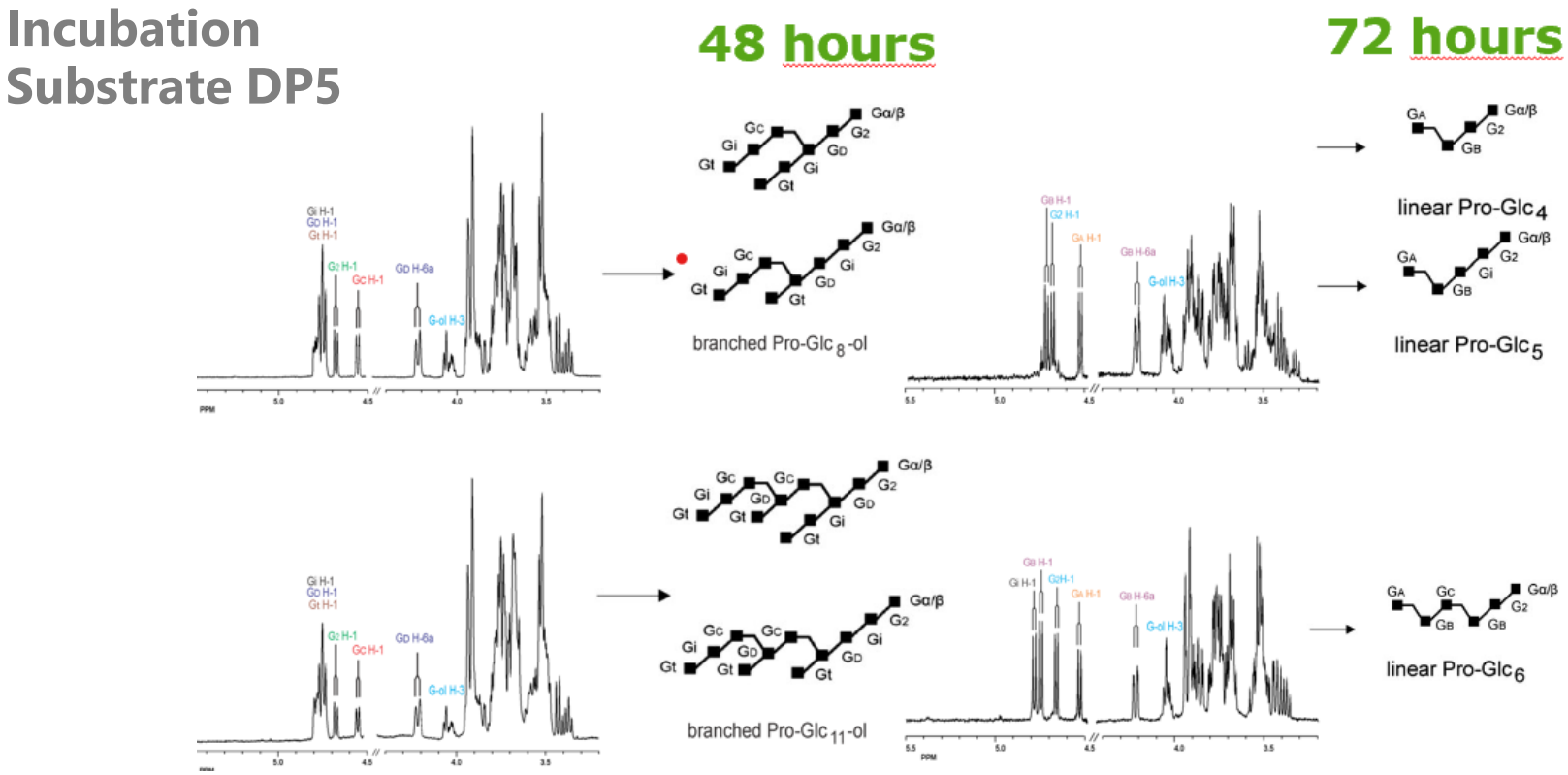
Substrate	Product
DP6	**DP9** (DP3 + DP6)

Substrate	Product
DP7	**DP10** (DP3 + DP7)

Substrate	Product
DP8	**DP11** (DP3 + DP8)

Substrate	Product
DP9	**DP12** (DP3 + DP7)

What type of linkages are formed?



Substrate: Laminarioligosaccharide DP5 (both donor and acceptor)

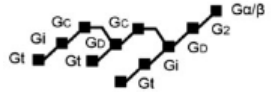
When donor is depleted, hydrolysis occurs, and initially formed branched products are being degraded into linear kinked products

after prolonged reaction kinked linear products are formed **26% internal β-1,6-linkages** are observed

Enzyme toolbox for for production of complex mixed linkage, kinked or highly branched, cyclic or linear β-glucans from laminarin polysaccharides, *in vitro* .

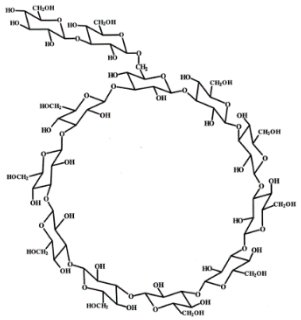
kinked

or branched



Matis has isolated and characterized a number of different transglucosidases active on beta-1,3 glucan polysaccharides such as laminarin.

or cyclic



A patent hasaquired acquired for the branching transglucosidase

Variety of beta- glucans having healthbenefits

Anti-inflammatory activity

Table 1. Examples of β-glucans with different structures, isolated from different natural sources (Stone and Clarke, 1992)

Type of β-glucan (structure description)	Natural source – trivial name of β-glucan
(1,3)-β-glucans (linear, homogeneous)	– bacterium *Alcaligenes faecalis* – curdlan – algae *Euglena gracilis* – paramylon – *Poria cocos* – pachyman – *Vitis vinifera* – callose – tamarack (*Larix laricina*) – laricinan
(1,3),(1,6)-β-glucans (linear with (1,6)-linked β-glucosyl side branches)	– algae *Laminaria sp.* – laminarin – *Claviceps purpurea* – wall glucan – *Sclerotinia sclerotiorum* – wall glucan
(1,3),(1,6)-β-glucans (linear with (1,6)-linked β-glucosyl or β-gentobiosyl side branches)	– brown algae *Eisenia bicyclis* – laminarin – mushroom *Lentinula edodes* – wall glucan
(1,3),(1,6)-β-glucans („branch on branch" structure)	– yeast *Saccharomyces cerevisiae* – cell wall glucan – mushroom *Schizophyllum commune* – wall glucan
(1,3),(1,4)-β-glucans (linear)	– cereal β-glucans – Iceland moss *Cetraria islandica* – lichenin
(1,3),(1,4)-β-glucans (linear with (1,4)-linked β-glucosyl side branches)	– oyster mushroom *(Pleurotus ostreatus)* – wall glucan

Enzymatic refining of polysaccharides from Brown macroalgae

Alginate

A polyuronate

Aim: cell free synthesis of a platform chemical, KDG

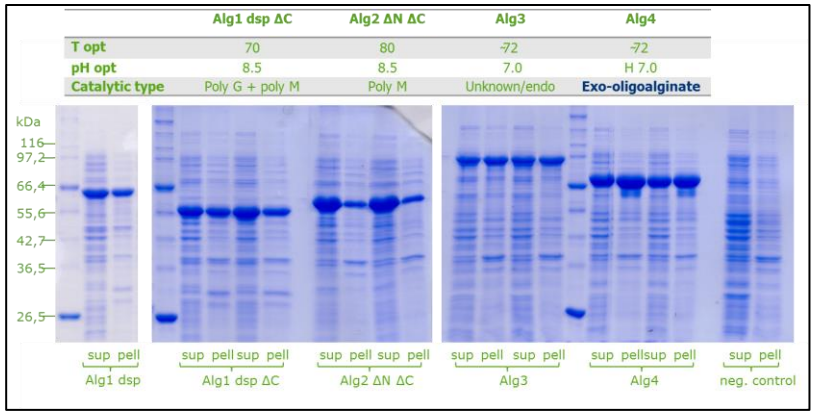
Alginate degradation

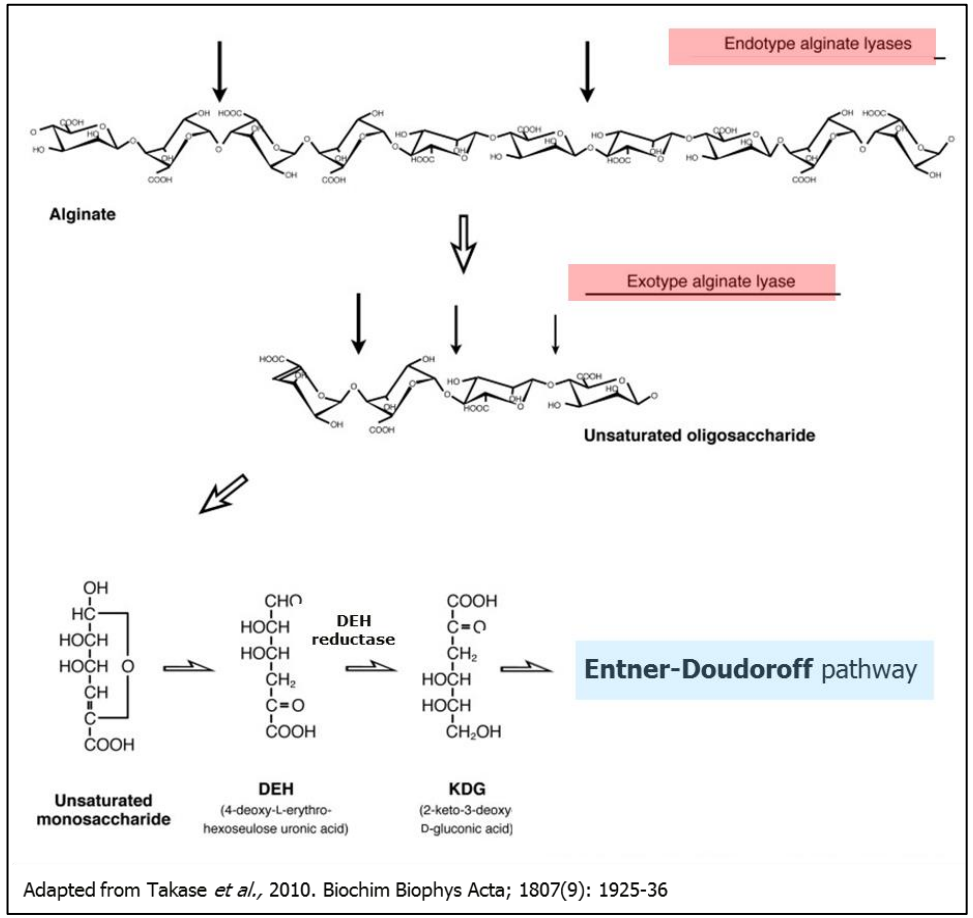
Matis has developed and expressed in high yield thermophilic alginate lyases for near complete degradation of alginate to unsaturated mono-uroantes/DEH

Avilable in different hosts

- *E. coli*
- *B. subtilis*
- *Lactobacillus reuterii*

For preprocssing of macroalgal biomass

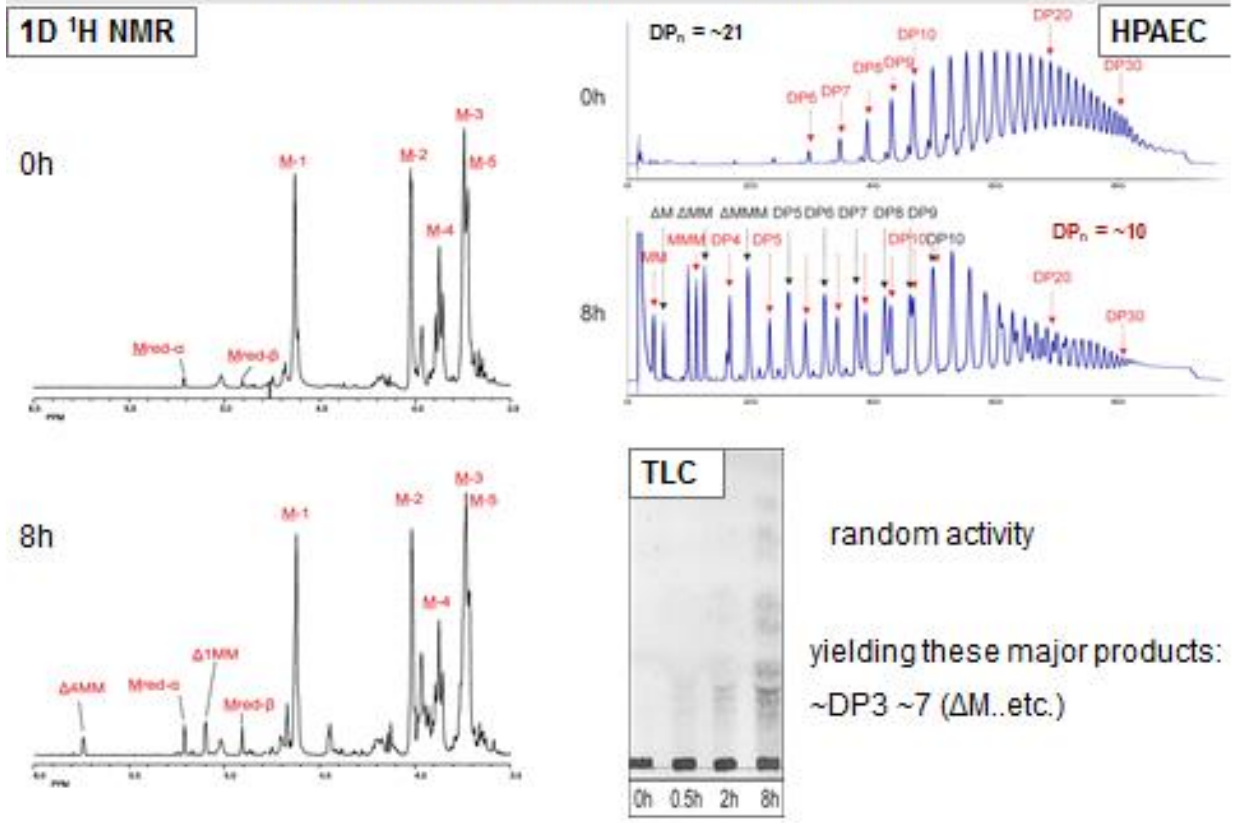




Adapted from Takase *et al.*, 2010. Biochim Biophys Acta; 1807(9): 1925-36

Alg3: an endo-type alginate lyase

Figure 17. Degradation pattern of AlgRm3 on M-block



random activity

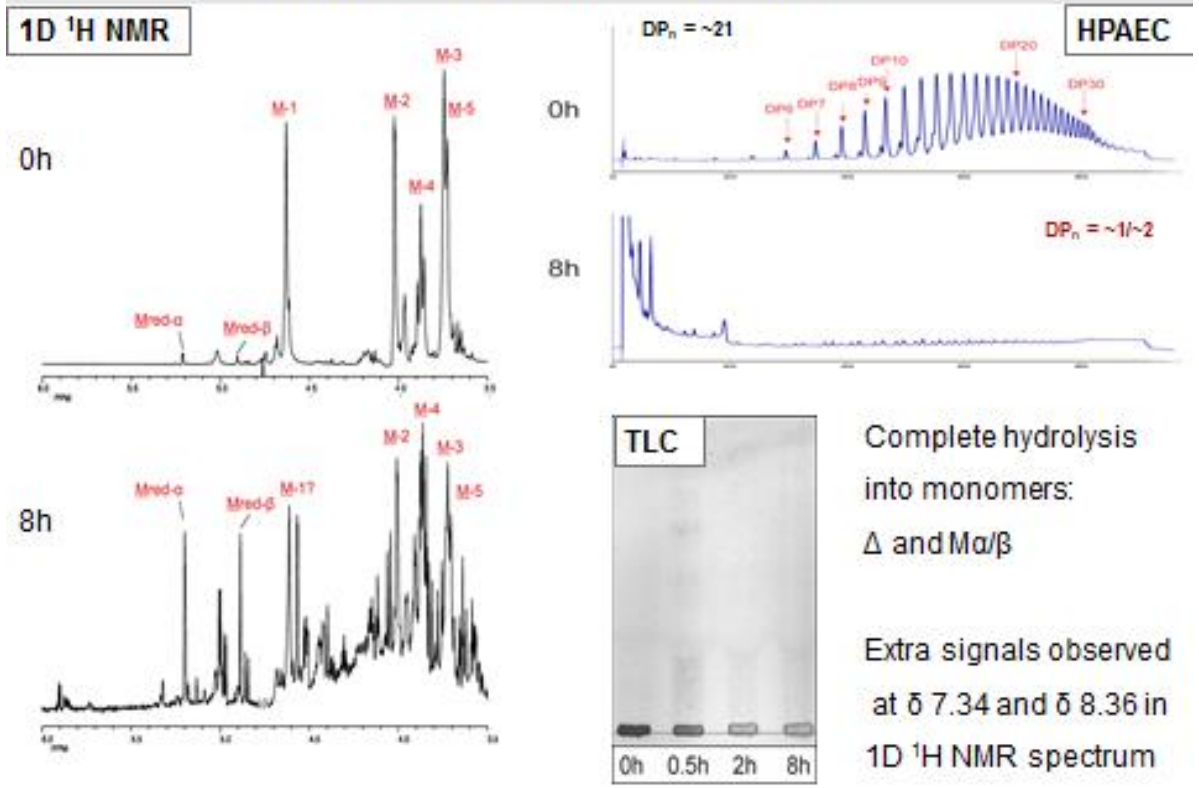
yielding these major products:

~DP3 ~7 (ΔM..etc.)

…M↓GMMM↓GGG↓GG↓GGGM↓MMMM↓MM↓GM↓GM↓GM↓GM↓MMM↓MMMM…

Alg4: an exo-type alginate lyase – oligo-alginate lyase

Figure 20. Degradation pattern of AlgRm4 on M-block

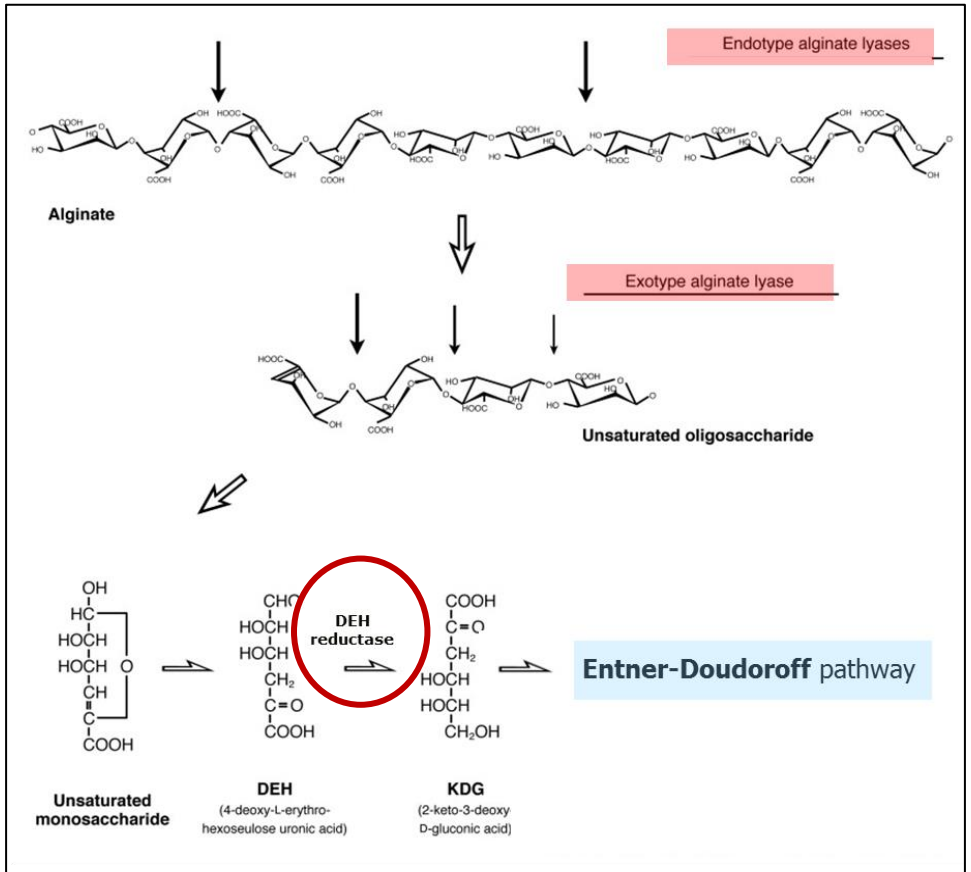


Complete hydrolysis into monomers: Δ and Mα/β

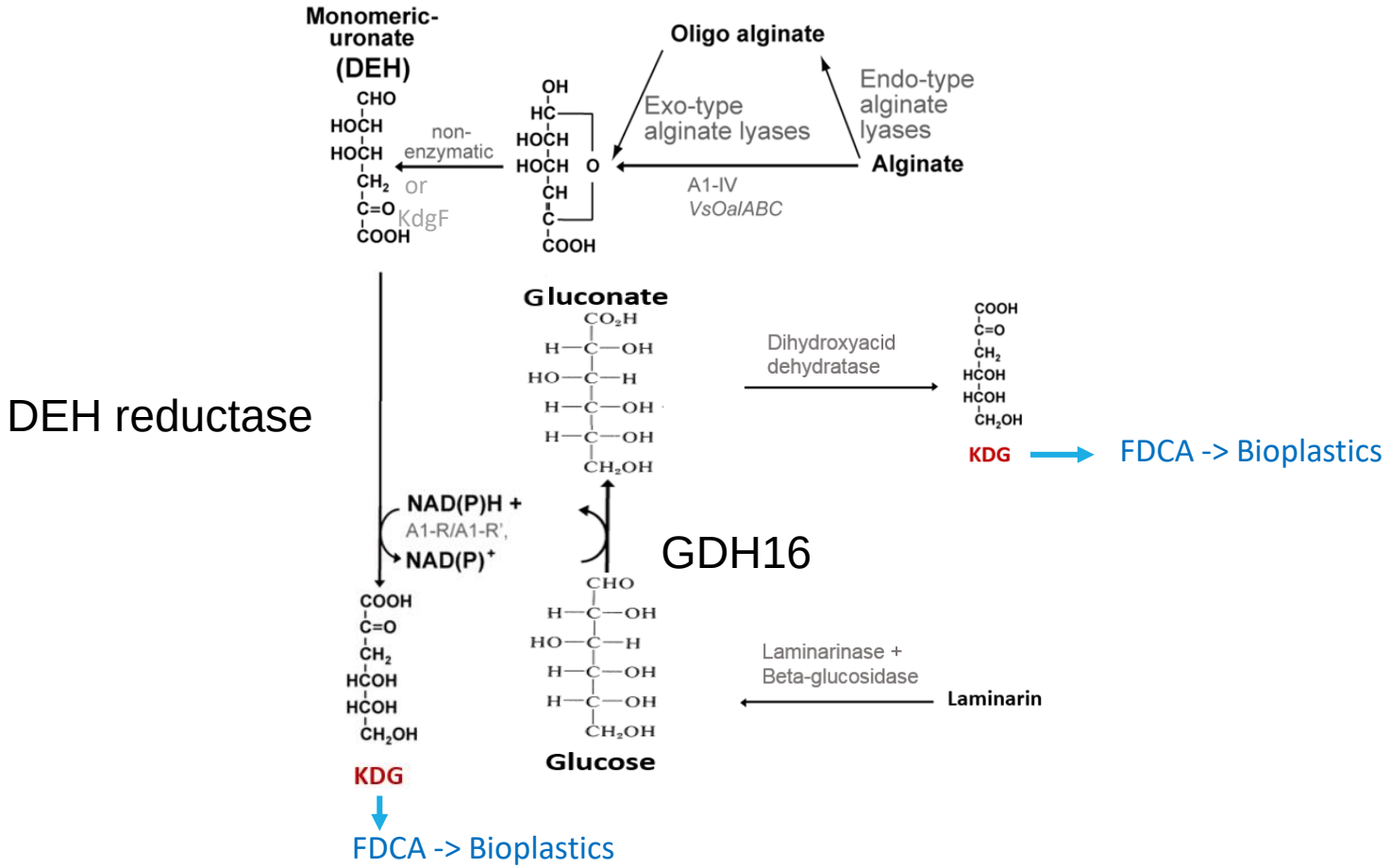
Extra signals observed at δ 7.34 and δ 8.36 in 1D ¹H NMR spectrum

KDG°from aginate only

- Celf free synthesis of platform chemcials and building
- blocks from metabolites of alginate catabolism

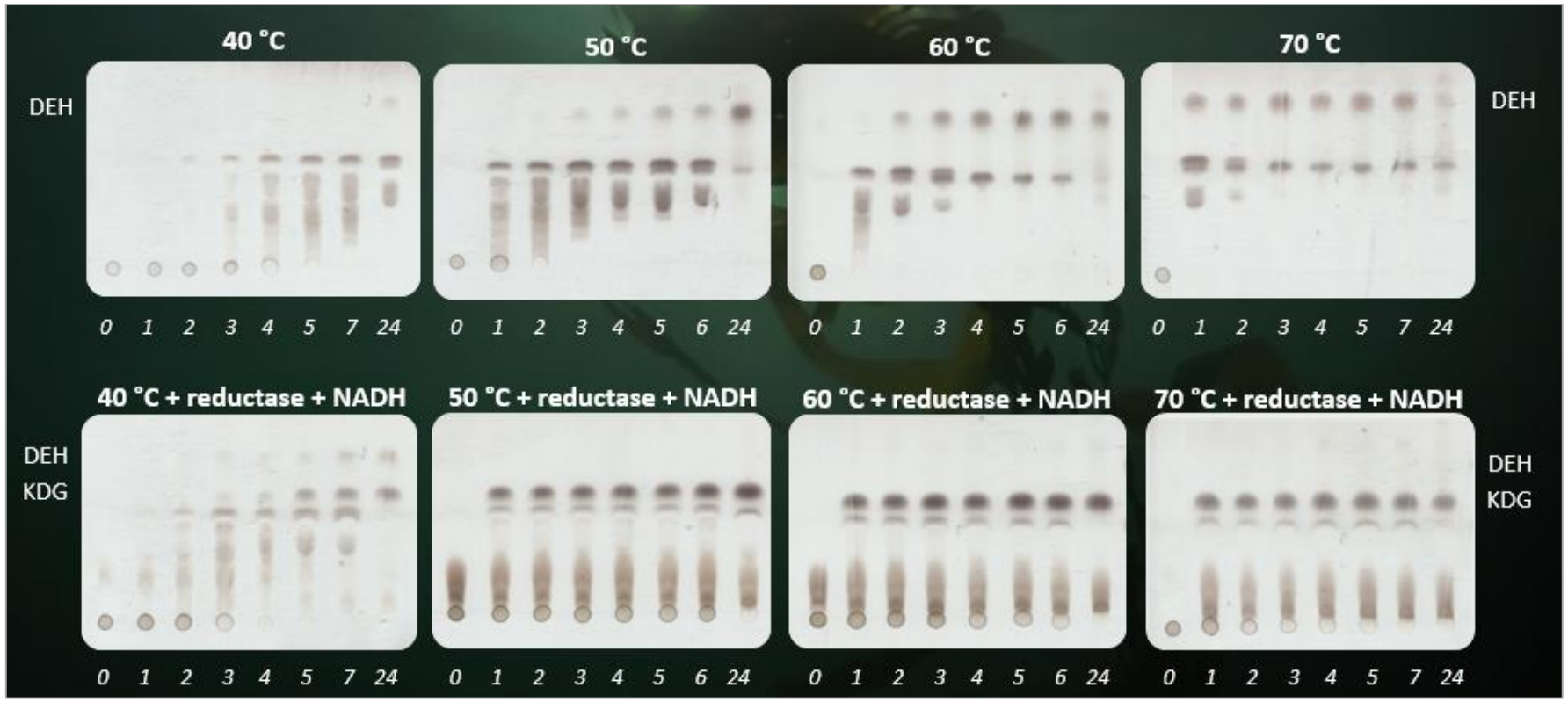


One-pot synthesis of KDG from alginate and laminarin



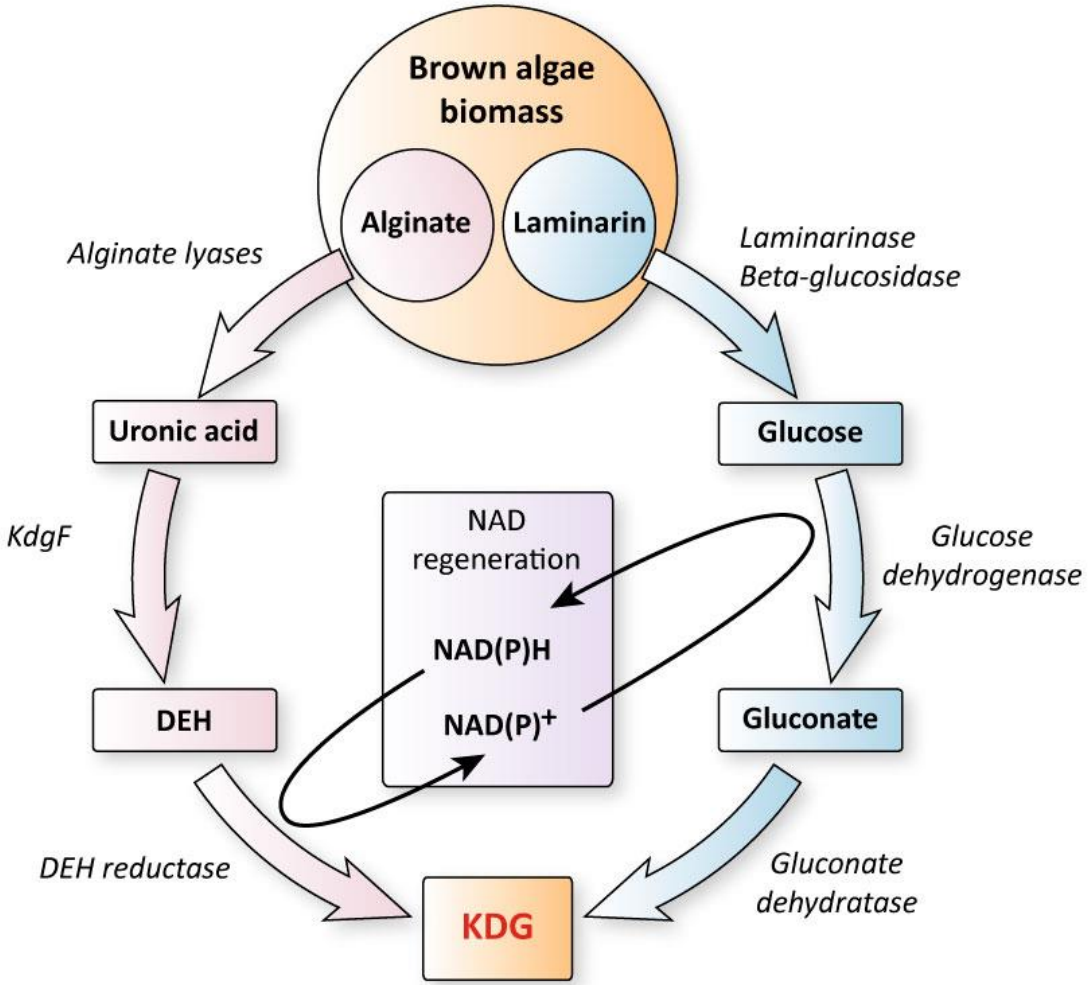
Temperature range

Alginic acid digestion (top row): **DEH production, bottom row KDG producion**



Alginic acid digestion and DEH redcution (bottom row): **KDG production**

Alg3, Alg4 and Adh51 (DEH reductase)


Brown algae biomass
Alginate
Laminarin
Alginate lyases
Laminarinase
Beta-glucosidase
Uronic acid
Glucose
KdgF
NAD regeneration
NAD(P)H
NAD(P)+
Glucose dehydrogenase
DEH
Gluconate
DEH reductase
KDG
Gluconate dehydratase

- Thank you for your attention

Coworkers

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Dr. Frits van der Klis

Dr. Paulien Harmsen

,

Lund University

Prof. Eva Nordberg Karlsson

References

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