Supporting Information

Model-based complete enzymatic production of 3,6-anhydro-L-galactose, from red algal biomass

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Supplementary Table S1: Primers used in the study

Target	Primer Sequence 5’ – 3’
Aga 16B	
Aga 16B_F	5’-GGCGGTGGTGGCGGCATGCCTGCTGATAAAAACAGTGAT-3’
Aga 16B_R	5-’GTTCTTCTCCTTTGCGCCCCTACTGCACAGATCCCATTATTTTTG-3’
Aga 50D	
Aga 50D_F	5’-GGCGGTGGTGGCGGCATGTTATTCGATTTTGAAAACGATCAAGTCCCTTC-3’
Aga 50D_R	5’-GTTCTTCTCCTTTGCGCCCCTATTTGCTGCCTAGCCTTTCGGTGT-3’
ABG	
ABG (WSP)_F	5’-GGCGGTGGTGGCGGCATGGACTTAAGAATTACCTCATATTTTT-3’
ABG (WOS)_F	5’-GGCGGTGGTGGCGGCATGGCAGATTGGGACGGAATTCC-3’
ABG_R	5’-GTTCTTCTCCTTTGCGCCCCTAGTTGCTAAGCGTGAACTTATCTAGG-3’
NABH	
NABH_F	5’-GGCGGTGGTGGCGGCATGAGCGATTCAAAAGTAAATAAAAAATTG-3’
NABH_R	5’-GTTCTTCTCCTTTGCGCCCCTATACTGCTCCGGAATCGCCTG-3’

Supplementary Table S2: Bacteria strains used in this study

Name	Description	Reference
	Strains	
Saccharophagus degradans 2-40	*Saccharophagus degradans*, an agarolytic marine bacterium metabolizing agar as the sole carbon source	(Ekborg *et al.*, 2006)
E. coli DH5α	F^-, *endA1*, *supE44*, *thi-1*, *recA1*, *relA1*, *gyrA96*, *deoR*, nupG, Φ80d*lacZ*ΔM15, Δ (*lacZYA-argF*) U169, *hsdR17* (r_K^-, m_K^+) and λ^-	(Grant *et al.*, 1990)
E. coli BL21 (DE3)	F^- *ompT gal dcm lon hsdS*$_B$($r_B^-m_B^-$) λ(DE3 [*lacI lacUV5-T7p07 ind1 sam7 nin5*]) [*malB*$^+$]$_{K-12}$(λ^S)	Studier *et al.*, 1986)

Supplementary Table S3: Vectors used in this study

Name	Description	Source
	Plasmids	
pB3/His	Modified pET21a vector; insertion of an LIC sequence with a N-terminus TEV cleavage site and His-tag	Structural Genomics Center, UC Berkley
pB4/MBP/His	Modified pET21a vector; insertion of an LIC sequence with a N-terminus TEV cleavage site, His-tag, and maltose-binding protein fusion tag	Structural Genomics Center, UC Berkley
pB6/MBP/His	Modified pET21a vector; insertion of an LIC sequence with a N-terminus TEV cleavage site, His-tag, and thioredoxin fusion tag	Structural Genomics Center, UC Berkley

Supplementary Data S1: Rate equations derived for constructing the kinetic model

DPn, agar polymer; DPo, neoagarooligosaccharides; DP3, agarotriose; DP2, neoagarobiose;

AHG, 3,6-Anhydro- L-galactose and Gal, D-galactose.

k_1 – Rate constant for reaction catalyzed by Aga16B (Agar polymer as substrate),

k_2 – Rate constant for reaction catalyzed by Aga50D (Agar polymer as substrate),

k'_2 - Rate constant for reaction catalyzed by Aga50D (Neoagarooligosaccharides as substrate),

k_3 – Rate constant for reaction catalyzed by ABG (Agarotetrose as substrate),

k_4 – Rate constant for reaction catalyzed by α-NABH (Neoagarobiose as substrate).

Rate equations derived for the two-enzyme route (E2)

$$\frac{d[DPn]}{dt} = -k_2 \times DPn$$

$$\frac{d[DP2]}{dt} = -k_2 \times DPn - k_4 \times DP2$$

$$\frac{d[Gal]}{dt} = -k_4 \times DP2$$

$$\frac{d[AHG]}{dt} = -k_4 \times DP2$$

Rate equations derived for the three-enzyme route (E3)

$$\frac{d[DPn]}{dt} = -k_1 \times DPn - k_2 \times DPn$$

$$\frac{d[DPo]}{dt} = k_1 \times DPn - k'_2 \times DPo$$

$$\frac{d[DP2]}{dt} = k_2 \times DPn + k'_2 \times DPo + k_2 \times DPo - k_4 \times Dp2$$

$$\frac{d[Gal]}{dt} = -k_4 \times DP2$$

$$\frac{d[AHG]}{dt} = -k_4 \times DP2$$

Rate equations derived for the four-enzyme route (E4)

$$\frac{d[DPn]}{dt} = -k_1 \times DPn - k_2 \times DPn$$

$$\frac{d[DPo]}{dt} = k_1 \times DPn - k_2' \times DPo$$

$$\frac{d[DP3]}{dt} = k_1 \times DPn + k_2' \times DPo - k_3 \times DP3$$

$$\frac{d[DP2]}{dt} = k_2 \times DPn + k_2' \times DPo + k_3 \times DP3 - k_4 \times Dp2$$

$$\frac{d[Gal]}{dt} = -k_4 \times DP2 + k_3 \times DP3$$

$$\frac{d[AHG]}{dt} = -k_4 \times DP2$$

Supplementary Data S2: Methods used for determining the rate constants

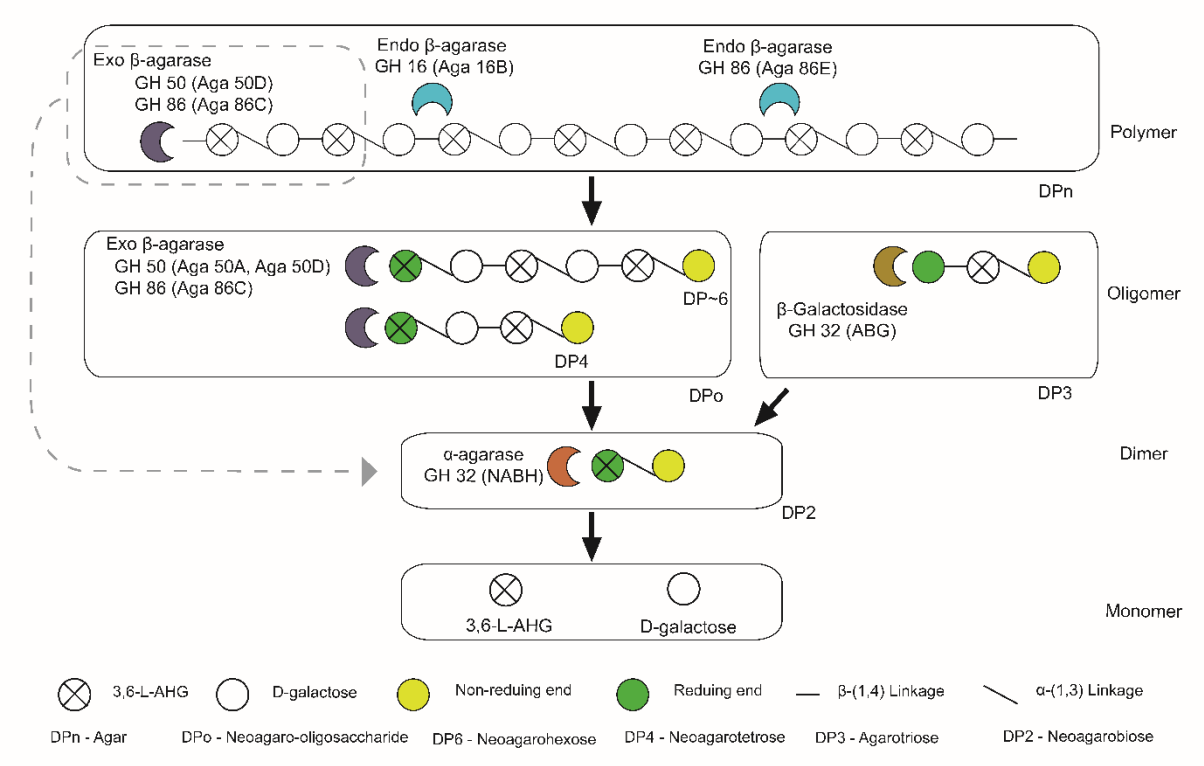
k_1 (rate constant for reaction catalyzed by Aga16B) and k_2 (rate constant for reaction catalyzed by Aga50D) were determined using 2% (w/v) molten agar [Bacto agar (cat. No 214010), BD Biosciences, San Jose, CA] in 20 mM Tris-Cl (pH 6.0) as the substrate. Enzymatic reactions were carried out at 55 °C and enzymes were inactivated by heating 5 minutes at 90 °C. Product concentration was determined by DNS assay using D-galactose as the standard.

k'_2 (rate constant for reaction catalyzed by Aga50D using neoagarooligosaccharides as the substrate) was determined using a mixture of neoagarooligosaccharides. According to our kinetic model Aga50D will act on both agar and neoagarooligosaccharides resulting from the hydrolysis of agar by Aga16B. These neoagarooligosaccharides have a degree of polymerization of 4 ~ 12. To obtain this substrate, 2% (w/v) molten agar [Bacto agar (cat. No 214010), BD Biosciences, San Jose, CA] in 20 mM Tris-Cl (pH 6.0) was hydrolyzed with Aga16B and the resulting neoagarooligosaccharides were quantified by DNS assay. 1% (w/v) neoagarooligosaccharide mixture in 20 mM Tris-Cl (pH 6.0) was reacted with Aga50D at 55 °C and enzymes were inactivated by heating 5 minutes at 90 °C. Product concentration was determined by DNS assay.

k_3 (rate constant for reaction catalyzed by ABG using agarotriose as the substrate) was determined as follows. According to our kinetic model ABG will act on agarotriose resulting from the hydrolysis of 2% (w/v) molten agar [Bacto agar (Cat. No 214010), BD Biosciences, San Jose, CA] in 20 mM Tris-Cl (pH 6.0) by Aga16B or Aga50D. But Aga16B or Aga50D also produces other products such as neoagarobiose, neoagarotetrose and neoagarohexose. But only the agarotriose are hydrolyzed by ABG. Therefore, we first react the hydrolysate of Aga16B and Aga50D for a prolonged time with excess amount of ABG to

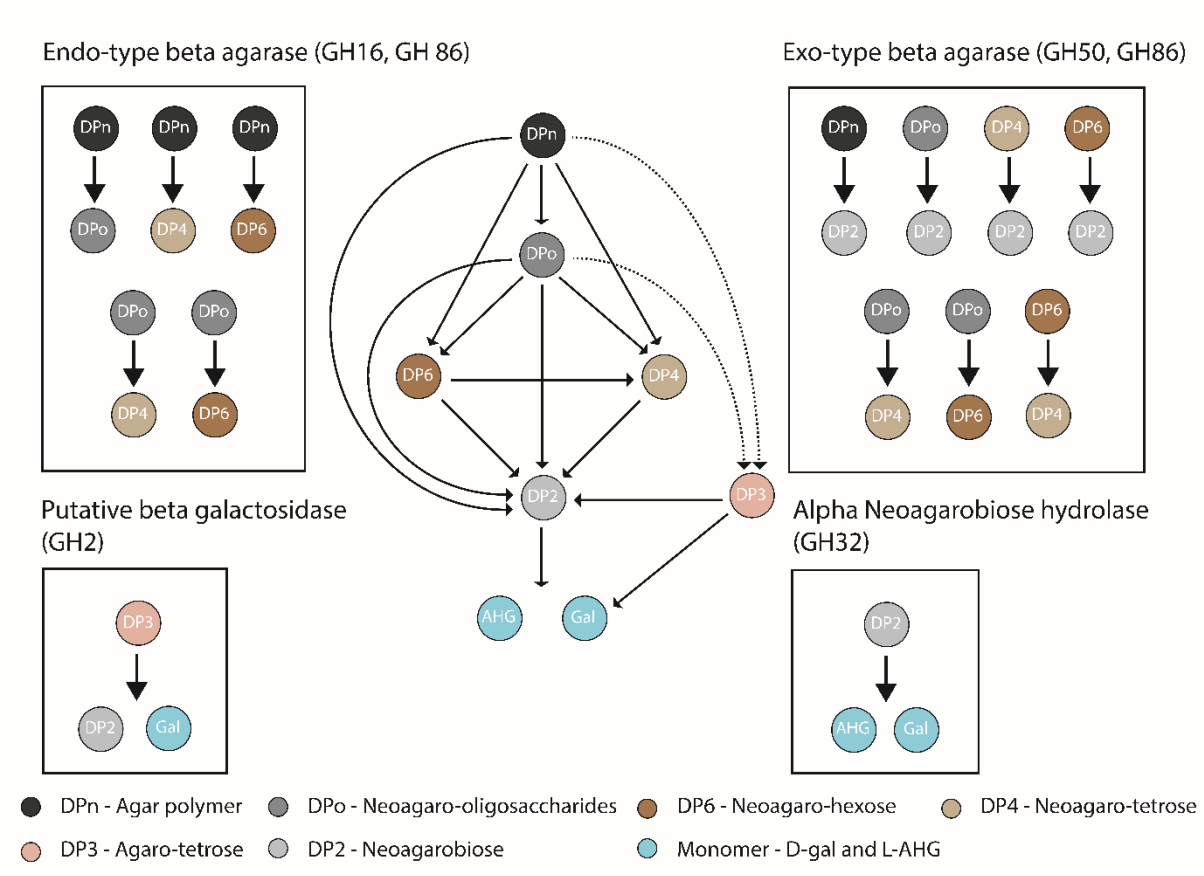
completely hydrolyze agarotriose. Concentration of agarotriose in the hydrolysate was determined by the increase in reducing sugars. 0.2% (w/v) agarotriose in 20 mM Tris-Cl (pH 6.0) was reacted with ABG at 55 °C and enzymes were inactivated by heating 5 minutes at 90 °C. Product concentration was determined by DNS assay.

k_4 (rate constant for reaction catalyzed by α-NABH using neoagarobiose as the substrate) was determined using neoagarobiose. According to our kinetic model α-NABH will act on α-NABH resulting from the hydrolysis of agar by Aga50D. To obtain this substrate, 2% (w/v) molten agar [Bacto agar (Cat. No 214010), BD Biosciences, San Jose, CA] in 20 mM Tris-Cl (pH 6.0) was hydrolyzed with Aga50D and the resulting neoagarobiose was quantified by DNS assay. 1% (w/v) neoagarobiose mixture in 20 mM Tris-Cl (pH 6.0) was reacted with α-NABH at 55 °C and enzymes were inactivated by heating 5 minutes at 90 °C. Product concentration was determined by DNS assay.



Supplementary Figure S1: Agarolytic pathway model of *Saccharophagus* degradans 2-40 elaborating the conversion of agar to D-galactose and L-AHG.

This model is based on the agarase enzymes found in *Saccharophagus* degradans 2-40, including two exo-type agarases belonging to the GH50 family, one exo-type agarase and one endo-type agarase belonging to the GH86 family, one endo-type agarase belonging to the GH16 family, an agarooligosaccharolytic β-galactosidase (ABG) belonging to the GH2 family, and neoagarobiose hydrolase (α-NABH) belonging to the GH117 family.



Supplementary Figure S2: Theoretical enzymatic paths for the conversion of agar polymer to monomers (D-galactose and L-AHG).

Endo-type β-agarase belonging to the GH16 and GH86, exo-type β-agarases belonging to the GH50 and GH86 families can collectively create 12 different theoretical enzymatic paths for the conversion of agar polymer to the disaccharide intermediate (neoagarobiose). In addition, agarooligosaccharolytic β-galactosidase (ABG) and α-neoagarobiose hydrolase (α-NABH) add two additional unique enzymatic paths.