

Supplementary Materials

Misincorporation of Galactose by Chondroitin Synthase of Escherichia Coli K4: From Traces to Synthesis of Chondburonan, a Novel Chondroitin-like Polysaccharide

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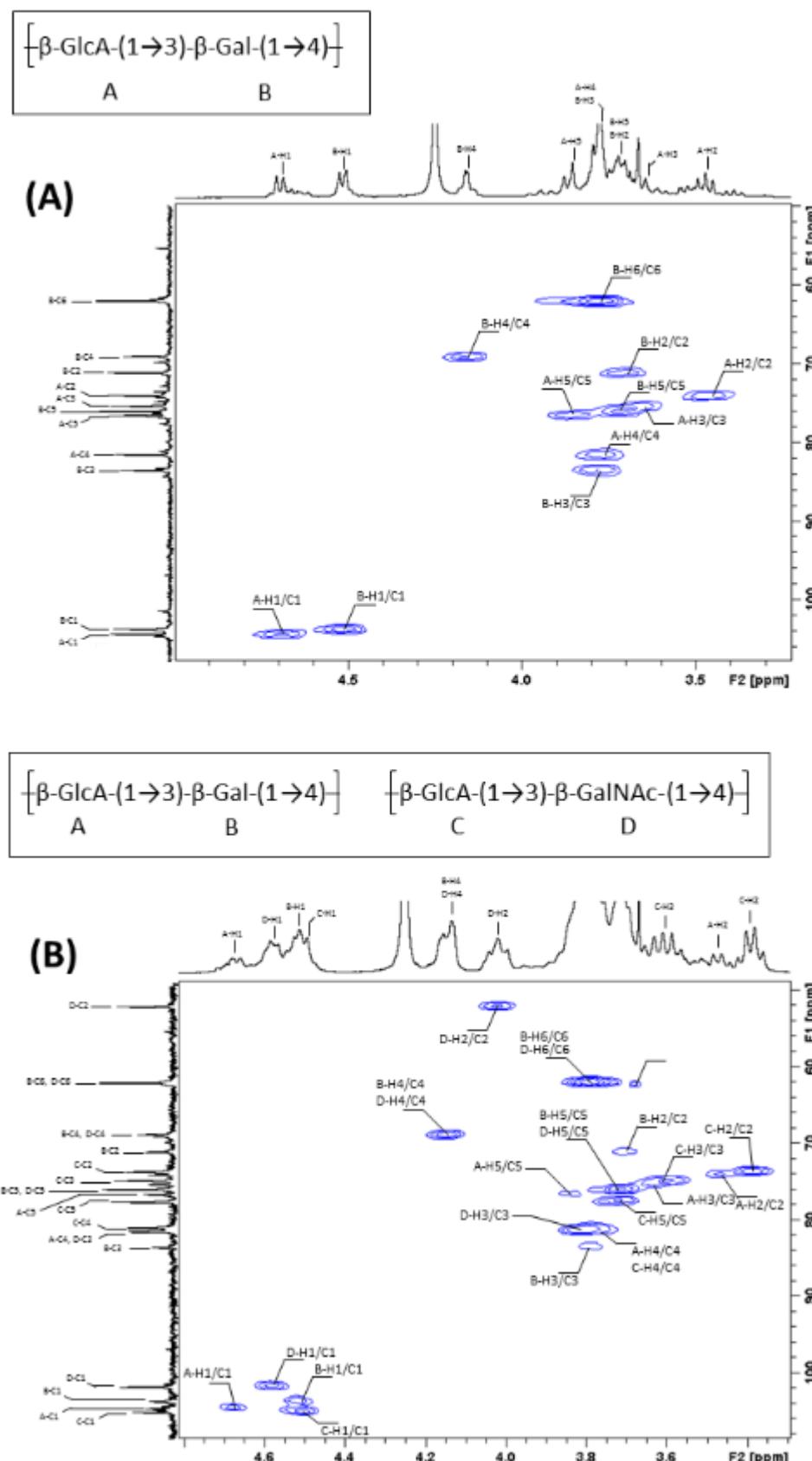
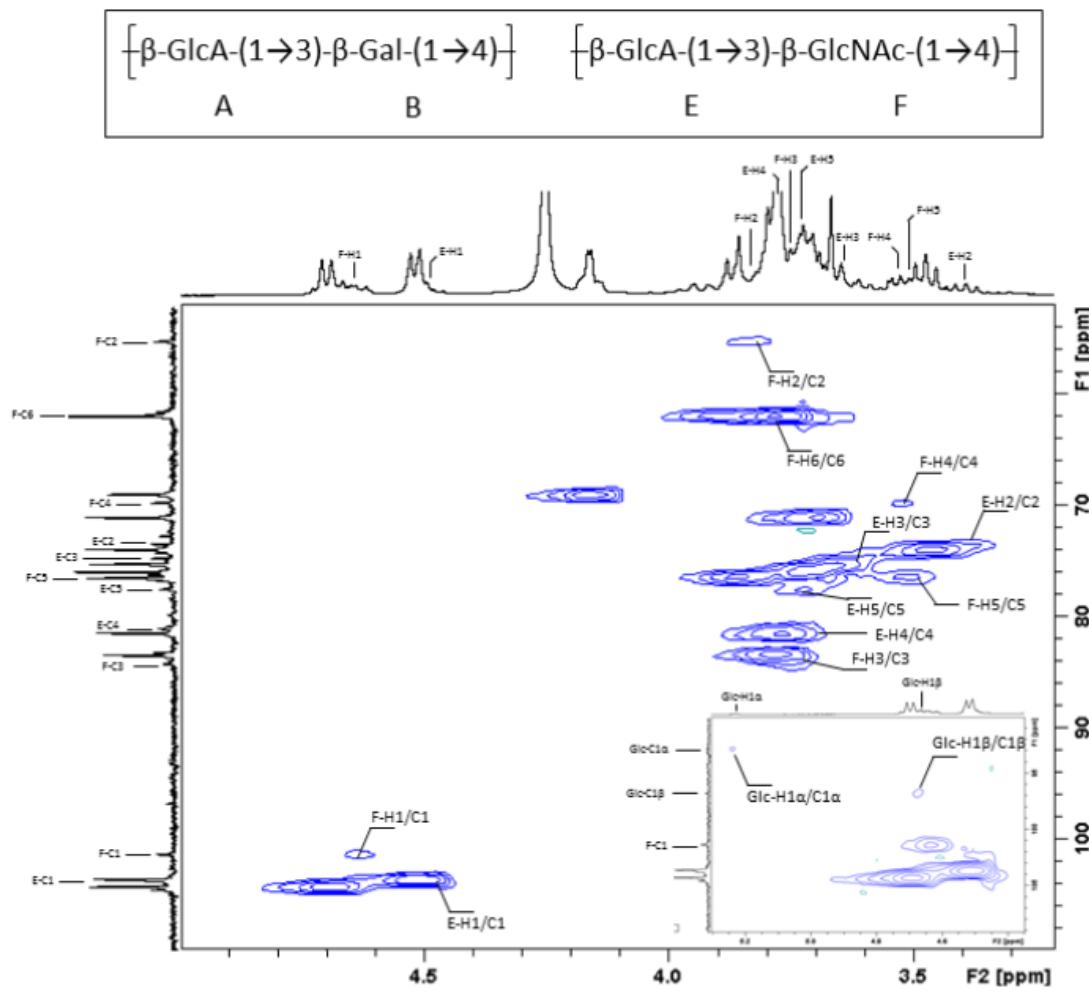


Figure S1. HSQC spectra of chondbiuronan (A) and chondroitin (B) obtained using 2% arabinose induction. Inset: chemical structure of the polysaccharides.



Sugar Residue	1	2	3	4	5	6 (6a,6b)	NHAc (CH ₃ ,CO)
Chondbiuronan EcDGCo							
E → 4)-β-D-GlcA-(1 →	¹ H 4.50	¹ H 3.39	¹ H 3.64	¹ H 3.78	¹ H 3.73	no	
	¹³ C 103.65	¹³ C 73.44	¹³ C 74.77	¹³ C 81.06	¹³ C 77.53	175.11	
F → 3)-β-D-GlcNAc-(1 →	¹ H 4.63	¹ H 3.84	¹ H 3.78	¹ H 3.52	¹ H 3.52	3.68, 3.93	2.04
	¹³ C 101.31	¹³ C 55.24	¹³ C 84.21	¹³ C 69.74	¹³ C 76.51	61.79	23.45, 175.67
→ 4)-α/β-D-Glc	¹ H 5.23/4.67	¹ H 3.59/3.30	nd	nd	nd	nd	
	¹³ C 92.7/96.8	nd	nd	nd	nd	nd	

Figure S2. HSQC spectrum of chondbiuronan showing the presence of Glc and GlcNAc incorporated in the polymer. Inset: chemical structure of the polysaccharides.

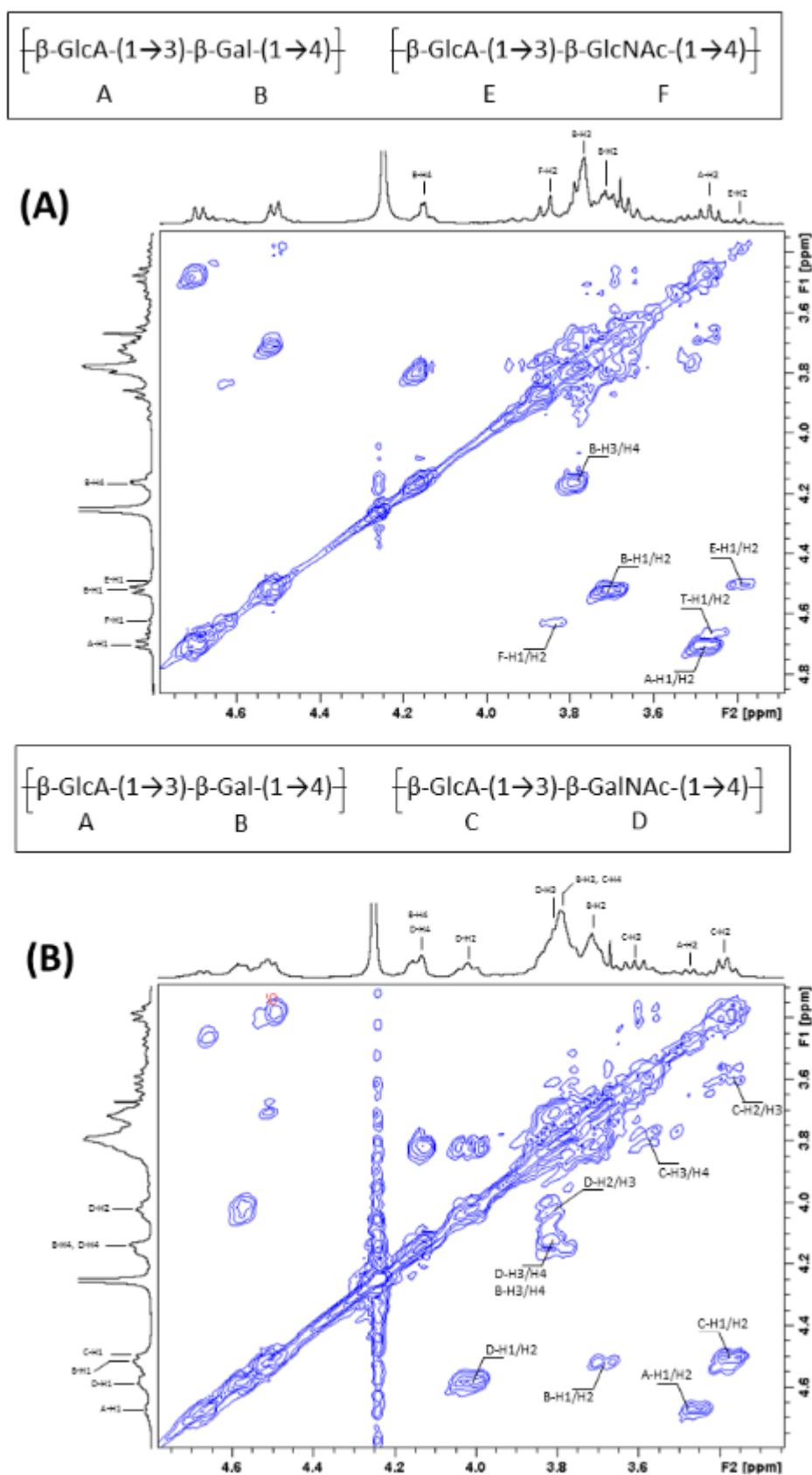


Figure S3. COSY spectra of chondbiuronan (A) and chondroitin (B) obtained using 2% arabinose induction. Inset: chemical structure of the polysaccharides.

Table S1. Genes, plasmids and *E. coli* strains used in this study.

Genes	Description	Ref. or source
GlcAT-P	Mouse glucuronyltransferase	GI 568960207
kfiD	UDP-glucose-6-dehydrogenase from <i>E. coli</i> K5	GI 735963
kfoA	UDP-GlcNAc 4-epimerase from <i>E. coli</i> K4	GI 21326786
kfoC	Chondroitin synthase from <i>E. coli</i> K4	GI 21326784
Plasmids		
pBBR1MCS3	Cloning vector, Tet, Plac promoter, bhr replicon	Kovach et al., 1995
pBAD33	Cloning vector, Cm, ParaBAD promoter, pSC101 replicon	Guzman, 1995
pBS	pBluescript II KS, Amp, colE1 replicon	Alting-Mees and Short, 1989
pBBR-glcATP-kfiD	pBBR1MCS3 expressing kfiD and GlcAT-P	Priem et al., 2017
pBS-kfoC	pBS expressing kfoC	Priem et al., 2017
pBAD-kfoA	pBAD33 expressing kfoA	This work
<i>E. coli</i> strains		
DH1	F-, endA1, gyrA96, thi-1, hsdR17(rK-,mK+), supE44, relA1	DSMZ
DJ	Strain DH1 lacA lacZ wcaJ	Yavuz et al., 2008
EcDGCo	Strain DJ, pBBR-glcAT-kfiD; pBS-kfoC	This work
EcDGCA	Strain DJ, pBBR-glcAT- kfiD; pBS-kfoC; pBAD-kfoA	This work