Supplemental material

The human gut microbe *Bacteroides thetaiotaomicron* encodes the founding member of a novel glycosaminoglycan-degrading polysaccharide lyase family PL29

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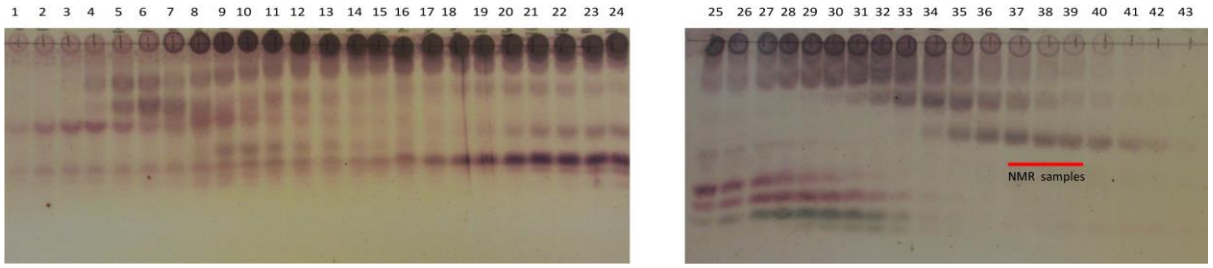
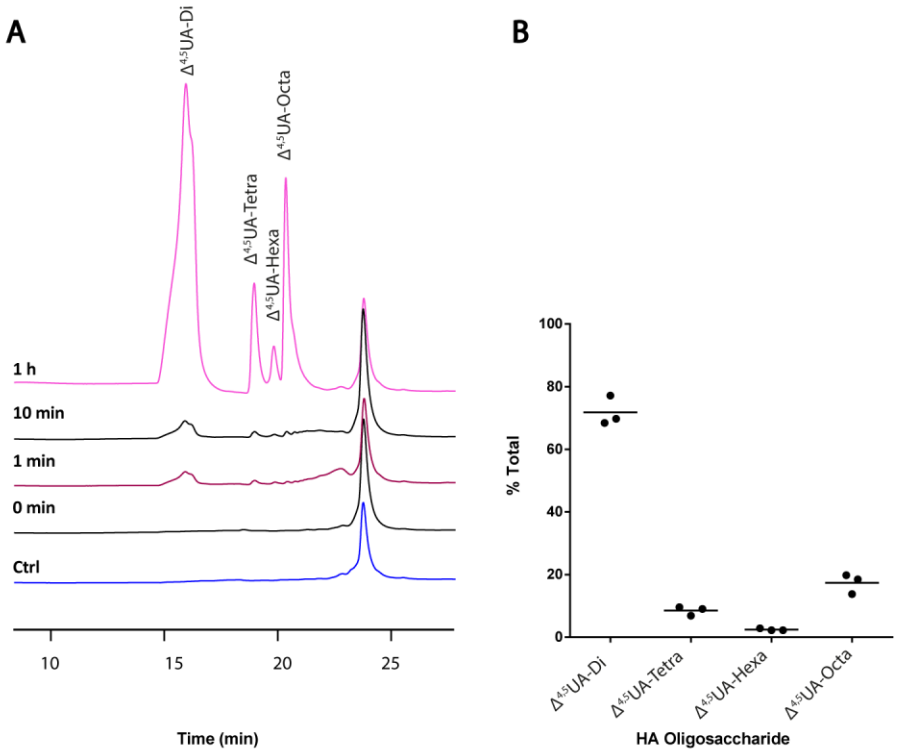
List of material included

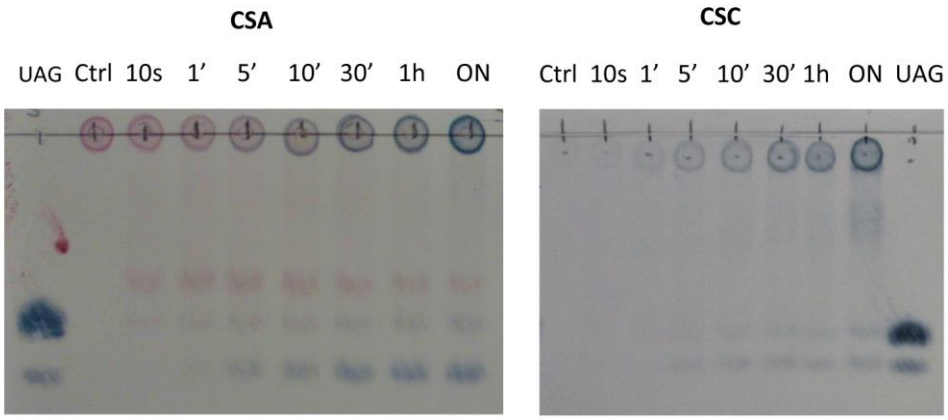
Supplemental figure 1. Purification and TLC analyses of end products of chondroitin 4-sulfate (CSA) digestion with BtCDH. CSA was digested overnight and end-products separated by size exclusion chromatography followed by TLC analyses. Aliquots from fractions 1-43 and 37-39 were separately pooled and used for NMR experiments.



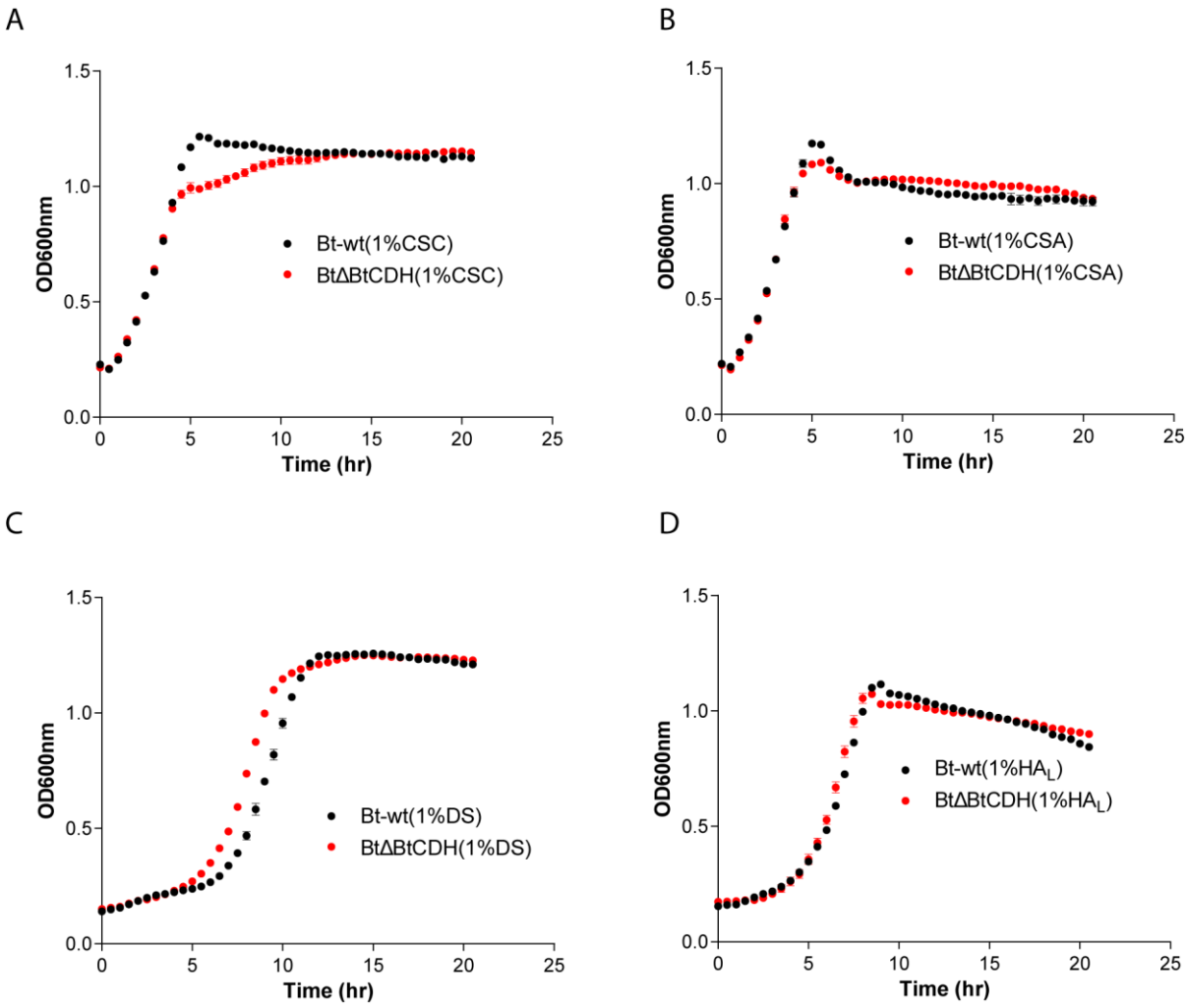
Supplemental figure 2. **HPAEC analyses of HA_L degradation by BtCDH**. **A)** HPAEC chromatogram of time course samples. HA_L (32 mg/ml) was incubated with 2 µM of BtCDH at 37 °C. Reactions were stopped at various time points indicated and 80 µg of digested HA analysed by HPAEC linked to a UV detector. **B)** Scatter plot showing the percentage of each product generated from the complete digestion of HA with BtCDH. Data was obtained by measuring the peak area for each product at the end of the reaction and expressing it as a percentage of the total. $\Delta^{4,5}$UA-Di: $\Delta^{4,5}$UA-disaccharide, $\Delta^{4,5}$UA-Tetra: $\Delta^{4,5}$UA-tetrasaccharide, $\Delta^{4,5}$UA-Hexa: $\Delta^{4,5}$UA-hexasaccharide, $\Delta^{4,5}$UA-Octa: $\Delta^{4,5}$UA-octasaccharide



Supplemental figure 3. Time course degradation of chondroitin sulfate by BtCDH lyase. For each sample, 13.3 mg/ml of substrate was treated with 0.3 µM of BtCDH. The reaction was stopped at various time points indicated and 53.4 ug of digested substrate analysed by TLC. UAG: $\Delta^{4,5}$UA-GalNAc disaccharide. Ctrl : Substrate without enzyme.



Supplemental figure 4. Growth curves of *B. thetaiotaomicron* WT and ΔBtCDH strains on various GAG substrates. Cells were grown in minimal medium containing 1% final concentration of each GAG substrate as sole sources of carbon. Bt-wt; *B. thetaiotaomicron* WT, BtΔBtCDH; *B. thetaiotaomicron* ΔBtCDH mutant, CSA; chondroitin sulfate from bovine trachea, DS; dermatan sulfate from porcine intestinal mucosa, CSC; chondroitin sulfate from shark cartilage and HA_L; Hyaluronanic acid sodium salt from *Streptococcus equi*



Supplemental figure 5: Analyses of culture supernatants of *B. thetaiotaomicron* WT, ΔBtCDH and BtCDHFLAG for secreted enzyme activity A) Analyses of supernatants by western blotting. Culture media at exponential phase growth in CSA were collected and centrifuged. The resulting supernatant (Sup1) was filtered to remove any remaining cells (Sup2) and later analysed by Western blotting using polyclonal rabbit anti-BtCDH antibodies. Lysates from whole cells (WC) and Sup2 samples concentrated ten times (10x Sup2) were also analysed. **B)** Comparison of growth supernatants from cultures of *B. thetaiotaomicron WT* (Sup_WT), ΔBtCDH (Sup_ΔBtCDH) and BtCDHFLAG (Sup_BtCDHFLAG) in CSA (10 mg/ml). Each supernatant (8 ul) at exponential phase alongside a control CSA medium (ME) which was not inoculated with any cells was analysed by TLC. **C)** Examination of growth supernatants for secreted enzyme activity. Supernatants (20 µl) obtained from cultures of *B. thetaiotaomicron WT* and ΔBtCDH in CSA were incubated overnight with 20 µl of 40 mg/ml CS substrate. The next day 4 µl of each sample was analysed by TLC.