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Characterizing carbohydrate degradation products from *Bacteroides thetaiotaomicron* to understand bacterial dextran utilization in the gut

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ABSTRACT

Purpose: Develop a derivatization-free analytical solution for the structural characterization of dextran degradation products from bacterial cultures.

Methods: Dextran standards were analyzed with ion chromatography coupled to mass spectrometry (IC-MS). Dextran samples were separated on a Thermo Scientific™ Dionex™ CarboPac™ PA300-4μm analytical column with a gradient of hydroxide and acetate eluents and detected sequentially by an electrochemical detector and a Thermo Scientific™ Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ Mass Spectrometer.

Results: IC-MS takes advantage of the supreme resolving power of high-performance anion exchange chromatography to separate dextran with varying degrees of polymerization, potentially separating dextran isomers as well. The Orbitrap MS facilitates the detailed structural identification and characterization of dextran, facilitating further characterization of the model for bacterial dextran import.

INTRODUCTION

Human health is intimately linked to the health of the gut microbiota and the metabolism of a diversity of colonic symbionts that help break down otherwise undigestible carbohydrates. Though we know of the importance of the bacteria colonizing the gut broadly, there is yet much to learn about the carbohydrate active enzymes that allow the bacteria to enact function.

Here we present a tool combining ion chromatography and high-resolution mass spectrometry for derivatization-free characterization of dextran. We apply this platform to analyze media supernatants from *Bacteroides thetaiotaomicron (Bt)* cultures to characterize dextran degradation as a function of both the molecular machinery available for dextran transport and the size of dextran available for metabolism.

MATERIALS AND METHODS

Sample Preparation

All dextran standards were purchased from Biosynth Carbosynth (San Diego, CA) except for dextran 1, which was purchased from Pharmacosmos (Holbaek, Denmark). Dextran standards were dissolved in water at 100 mg/L concentrations for the IC-MS analysis.

Bacterial cultures were grown until mid-exponential in a minimal media with 0.5% dextran as a sole carbon source. Culture supernatant was filtered, and metals were removed by treatment with Chelex resin. Samples for IC-MS analysis were dried in a SpeedVac concentrator and subsequently redissolved in water. The resulting solutions were purified with a Thermo Scientific™ HyperSep™ Hypercarb™ filter plate.

Test Method

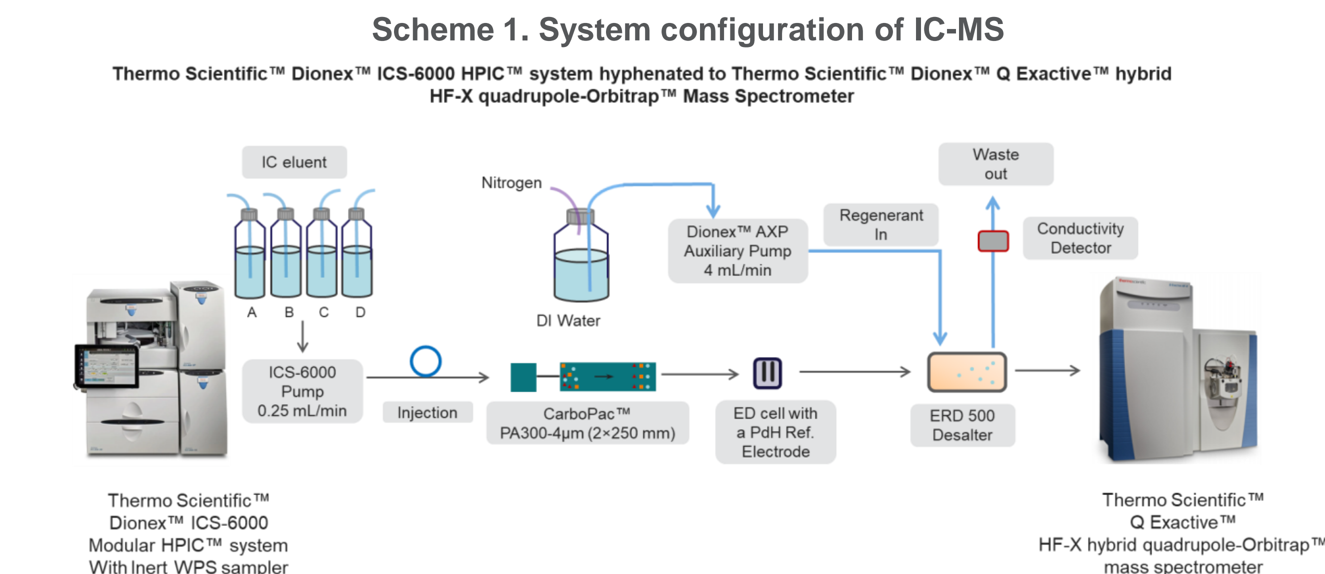
Dextran was analyzed using a Dionex™ ICS-6000 HPIC™ system outfitted with an electrochemical detector operated in the pulsed amperometric detection (PAD) mode and a Q Exactive™ HF-X hybrid quadrupole-Orbitrap™ mass spectrometer in a sequential configuration. The system configuration is shown in scheme 1. Dextran was separated on Dionex™ CarboPac™ PA300-4μm analytical and guard columns using sodium hydroxide and sodium acetate. Prior to MS injection, the column effluent was passed through a Thermo Scientific™ Dionex™ ERD 500-2 mm electrolytically regenerated desalter for salt removal. The ERD500-2 mm desalter was operated at 380 mA and regenerated with water at 4 mL/min.

The desalter effluent was introduced by a Thermo Scientific™ Heated Electrospray Ionization (HESI-II). Probe was operated in negative ionization mode. The spray voltage was 3.2 kV, and the capillary temperature was 320 ºC. The sheath and auxiliary gas flow rate were set to 40 and 20 arbitrary units, respectively. For MS experiment, the data were acquired across the scan range of *m/z* 400-2000. The AGC target was set to 10^6 with a minimum injection time of 60 ms and a resolution of 60,000 (FWHM at *m/z* 200). For MS^2, the AGC target was set to 10^5 with a maximum injection time of 300 ms and a resolution of 15,000. Five scans were performed at a normalized collisional energy of 28.

Data Analysis

Data were processed with Thermo Scientific™ Chromeleon™ Chromatography Data System and Thermo Scientific™ Xcalibur™ software. Precise oligosaccharide structures and MS/MS fragment ions were generated using GlycoWorkbench software [1]. All spectra were manually verified. Oligosaccharide structures were reported in accordance with Domon and Costello nomenclature [1].

Scheme 1. System configuration of IC-MS



RESULTS

To understand how *Bt* imports dextran for utilization, we knocked out a gene encoding a key endo-dextranase, GH66, and evaluated the bacteria's growth potential as a function of the size of dextran that was supplied as the sole carbon source. Figure 1 shows the dependence of *Bt* on GH66 for the import and utilization of dextran of larger size. Wildtype *Bt* grew regardless of dextran size, whereas *Bt* without GH66 grew substantially less when fed dextran of larger sizes.

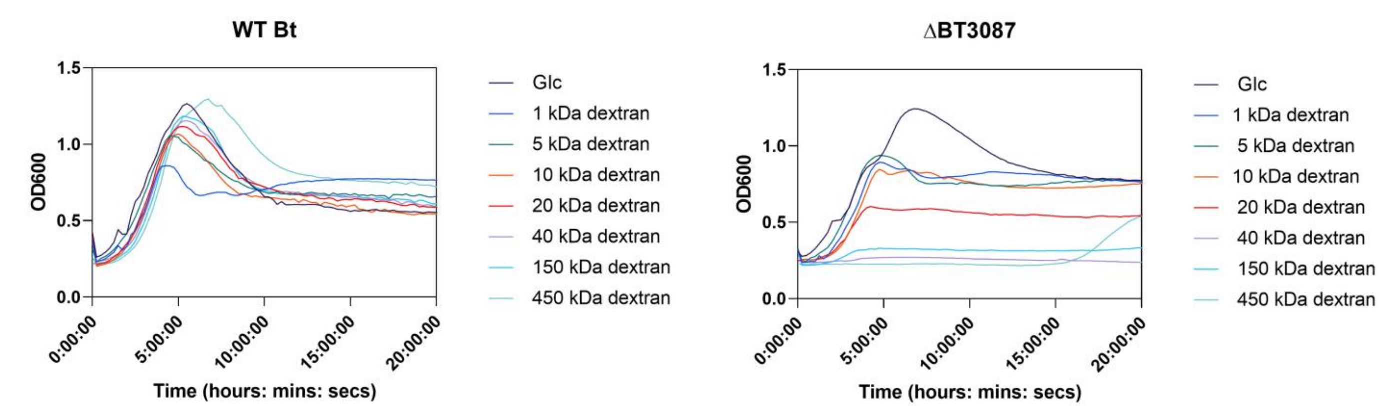


Figure 1. Growth curves of wildtype (WT) and GH66-knockout (ΔBT3087) *Bt* grown with dextran as a sole carbon source. Growth of knockout cells steeply falls off as the size of dextran increases.

Furthermore, we checked to see if there were any oligosaccharides left over in the culture supernatant after growing the bacteria by TLC (Figure 2). We found that that WT *Bt* can use all the dextran, leaving nothing in the culture supernatant after growth. Conversely, for the *Bt* knockout, we found that there is oligosaccharide content left with dextran as small as 3.5 kD. This indicates that the bacteria were unable to import the dextran, of these larger sizes, likely leading to the lack of growth observed in Figure 1 for the ΔBT3087 bacteria.

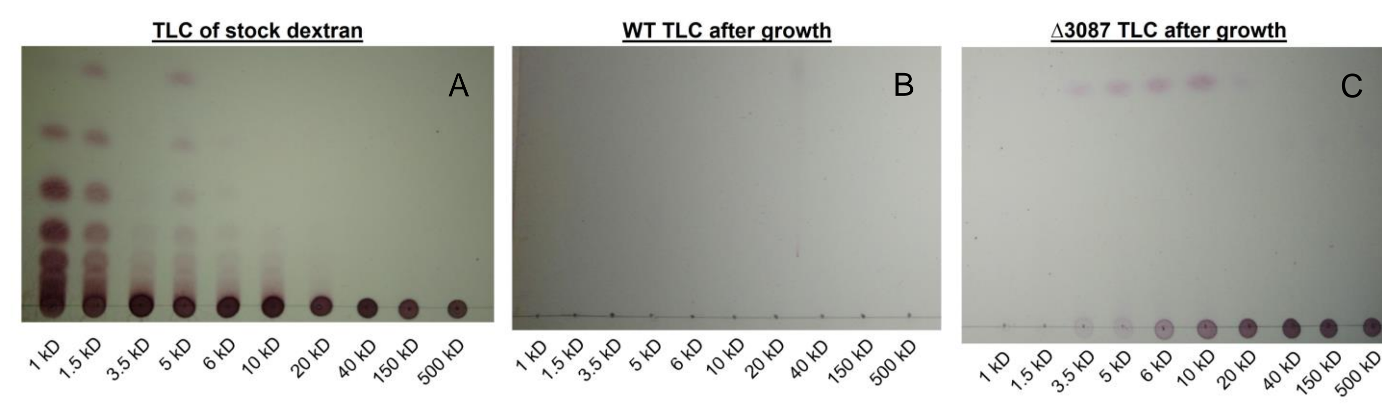


Figure 2. A) TLC data for stock dextran fed to bacterial cultures. B) Culture supernatant for WT *Bt* after growth. C) Culture supernatant for GH66 knockout *Bt* cells after growth. Spots remaining after growth indicate an inability to utilize dextran from stocks that were supplied as the sole carbon source during growth.

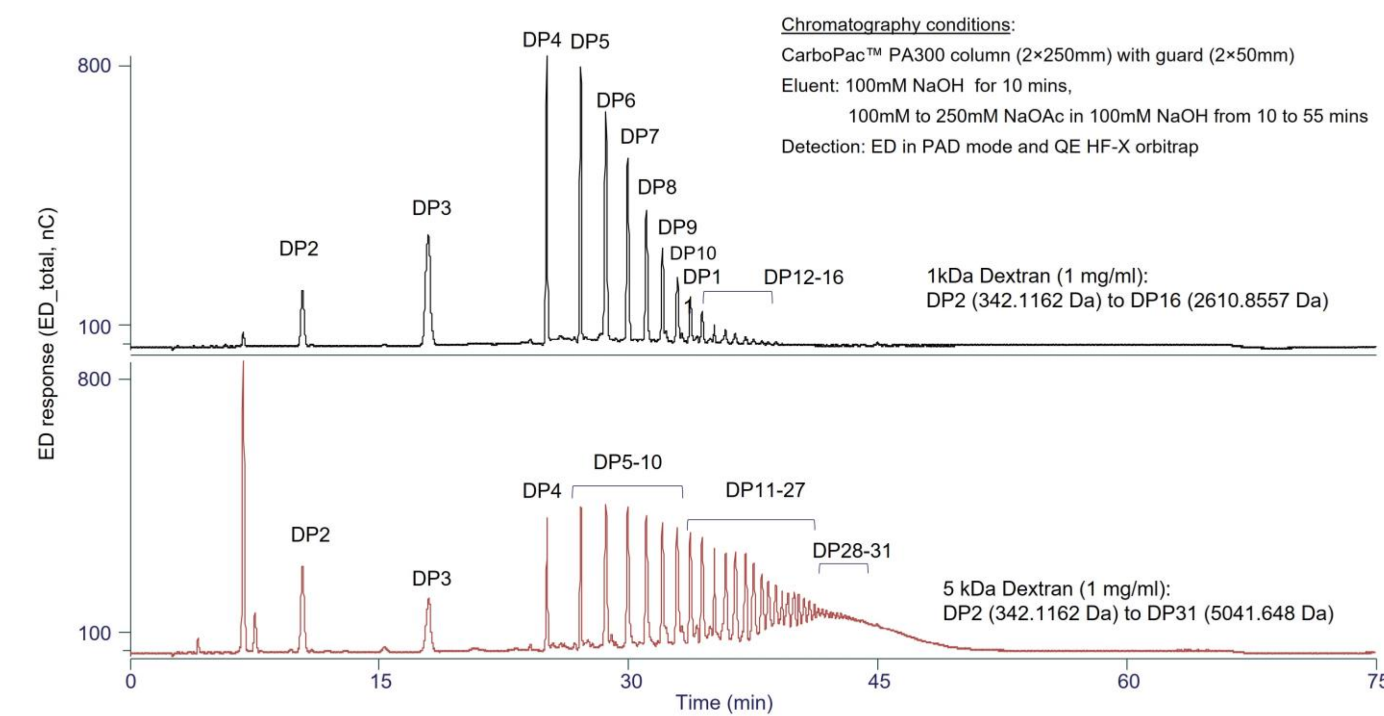
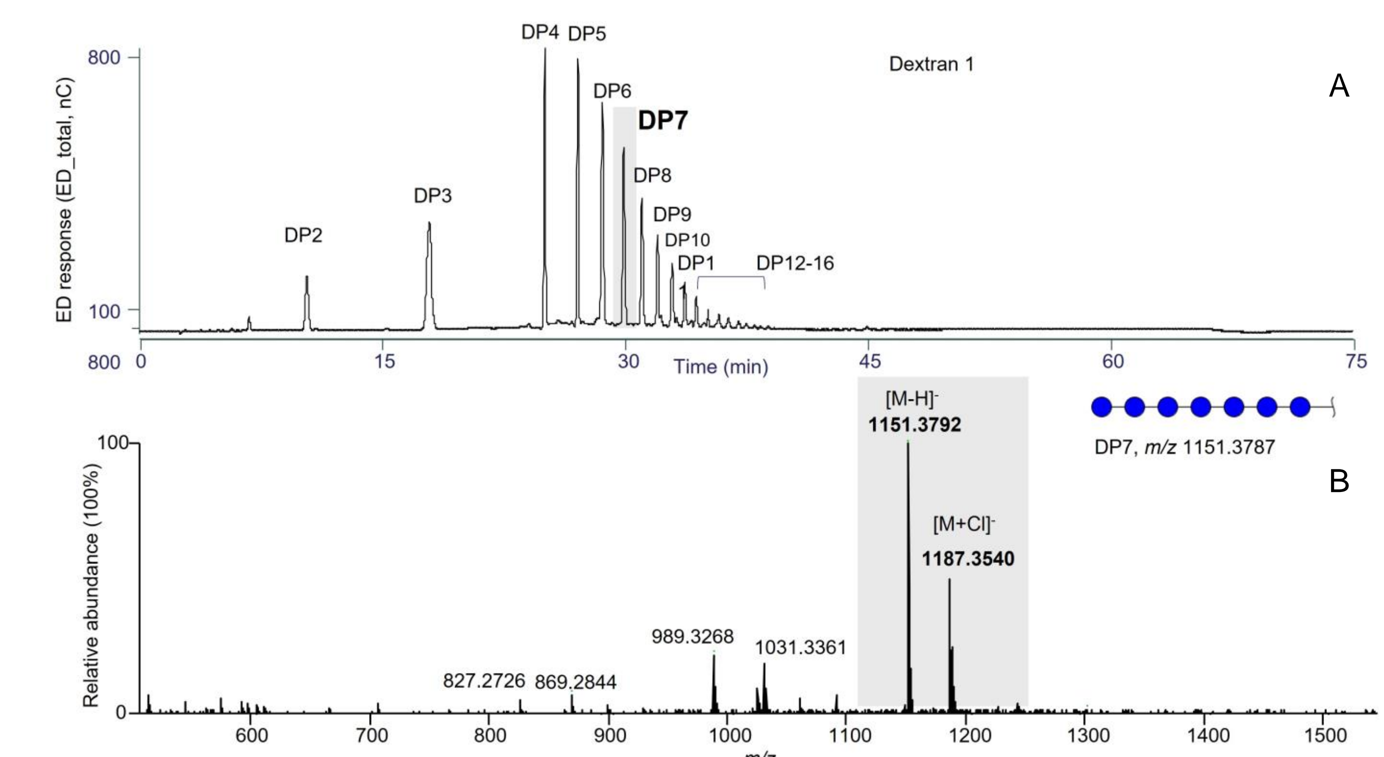


Figure 3. Chromatographic profiles of dextran 1, on the top, and dextran 5, on the bottom, acquired by electrochemical detection. Chromatographic conditions are listed in the upper right. DP values were assigned based on subsequent mass spectrometry analysis. DP; degrees of polymerization.



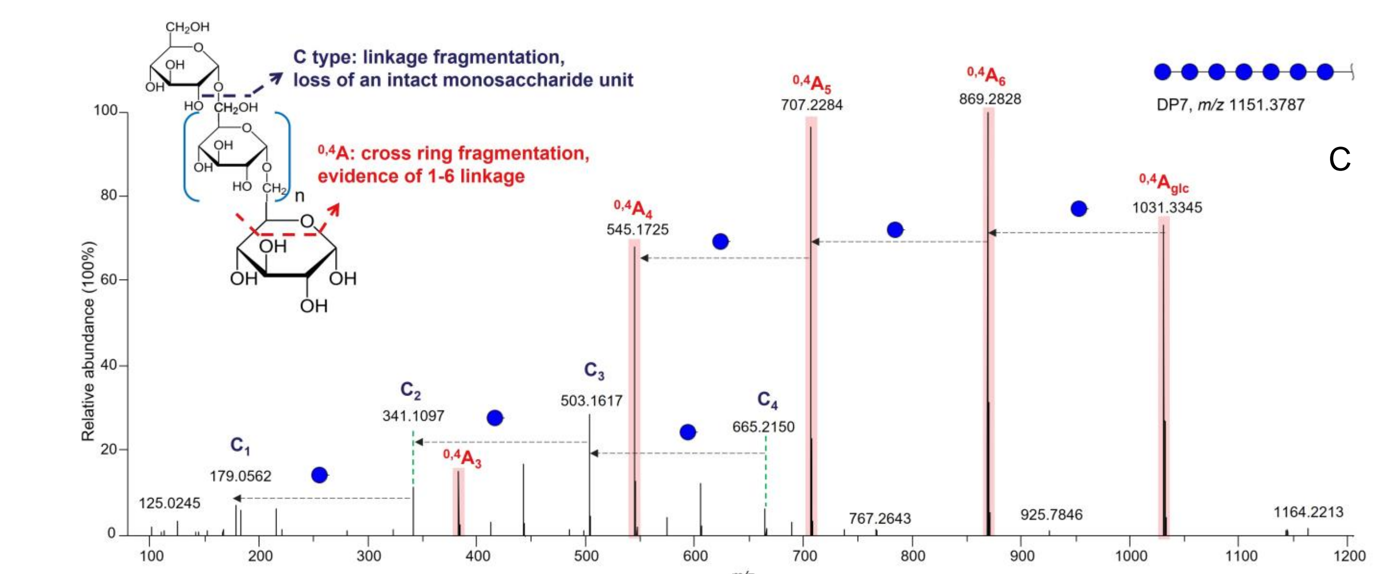


Figure 4. A) Chromatographic profile of dextran 1 obtained using the same chromatographic conditions as outlined in Figure 3. The highlighted peak corresponds to dextran with DP7. B) MS^1 spectrum of DP7 dextran. The highlighted region here shows the peaks corresponding to the intact mass of DP7 dextran and the intact mass with a chlorine adduct. C) Annotated MS^2 spectrum of DP7 dextran illustrating the types of glycan fragmentation that can occur. Using these data, we can assign a linear structure to the DP7 dextran.

Towards understanding the identify of the oligosaccharides remaining after growth of *Bt*, we first tested the performance of IC-MS on dextran standards. Figure 3 shows the ion chromatograms obtained from the analysis of dextran 1 and 5. In order to assign the chromatographic peaks with the corresponding DPs, we used high-mass accuracy MS detection downstream of the IC. Figure 4 illustrates how the MS^1 and MS^2 spectra enable the structural characterization of dextran using DP7 as an example. Having established the capabilities of IC-MS for dextran analysis, we next turned to the bacterial culture supernatants from WT and GH66-knockout *Bt*. Figure 5 shows the ion chromatograms for these culture supernatants. Subsequent MS analysis is underway to characterize the dextran that is left over from each of the culture supernatants.

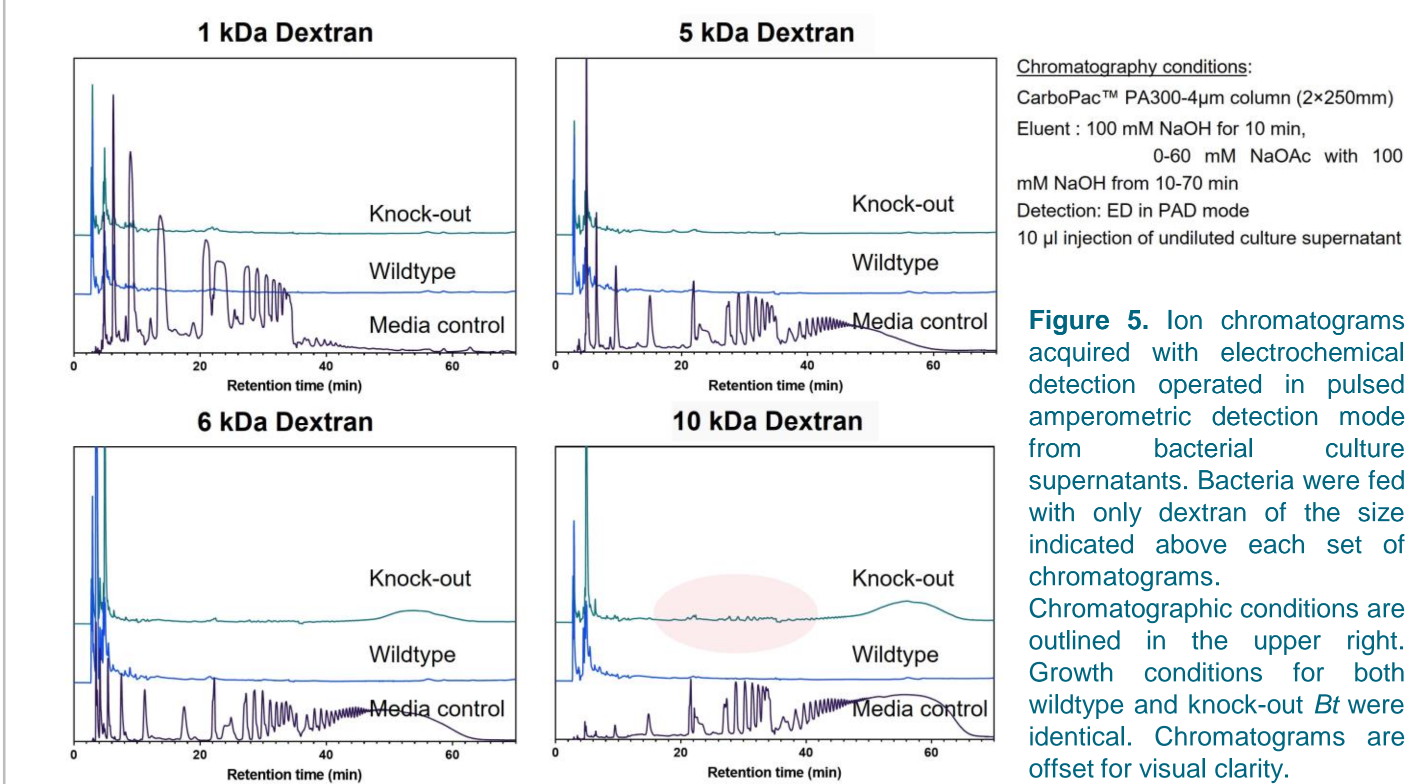


Figure 5. Ion chromatograms acquired with electrochemical detection operated in pulsed amperometric detection mode from bacterial culture supernatants. Bacteria were fed with only dextran of the size indicated above each set of chromatograms. Chromatographic conditions are outlined in the upper right. Growth conditions for both wildtype and knock-out *Bt* were identical. Chromatograms are offset for visual clarity.

CONCLUSIONS

- GH66 is essential for the import and utilization of dextran of larger sizes by *Bt*
- The CarboPac PA300-4μm column affords excellent separation of dextran samples over a wide range of degrees of polymerization
- IC-MS provides a powerful tool for the structural characterization of dextran of various sizes

REFERENCES

1. B. Domon, C.E. Costello. "A systematic nomenclature for carbohydrate fragmentations in FAB MS/MS spectra of glycoconjugates." Glycoconj. J. 5 (1988): 397–4.

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING

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