Drink to your health: Determining the digestive fate of putative prebiotics in beer

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BEER, CHEESE and GOOD COMPANY

Johansen *et al.* Food buying habits of people who buy wine or beer: cross sectional study. *BMJ* **2006**, *332* (7540), 519-22.

Wholesomeness of beer

<u>Premise</u>:

Commercially available beers contain low molecular weight oligosaccharides.

A sugar polymer containing 2-10 monosaccharide units

"Putative" Prebiotics

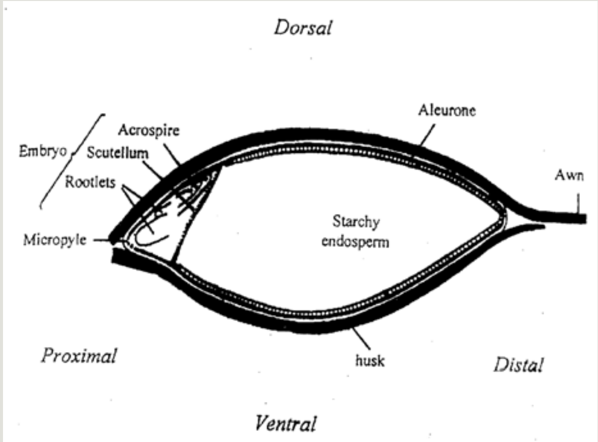
… "non-digestible food ingredient that beneficially affects the host by selectively stimulating growth and/or activity of 1 or a limited number of bacteria in the colon."

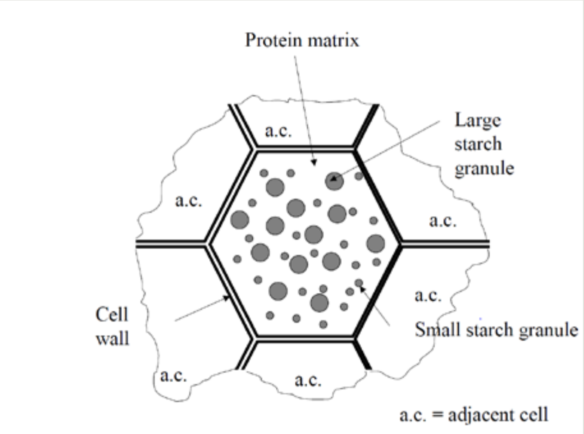
Gibson, G. R.; Roberfroid, M. B., Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of nutrition* **1995,** *125* (6), 1401-12.

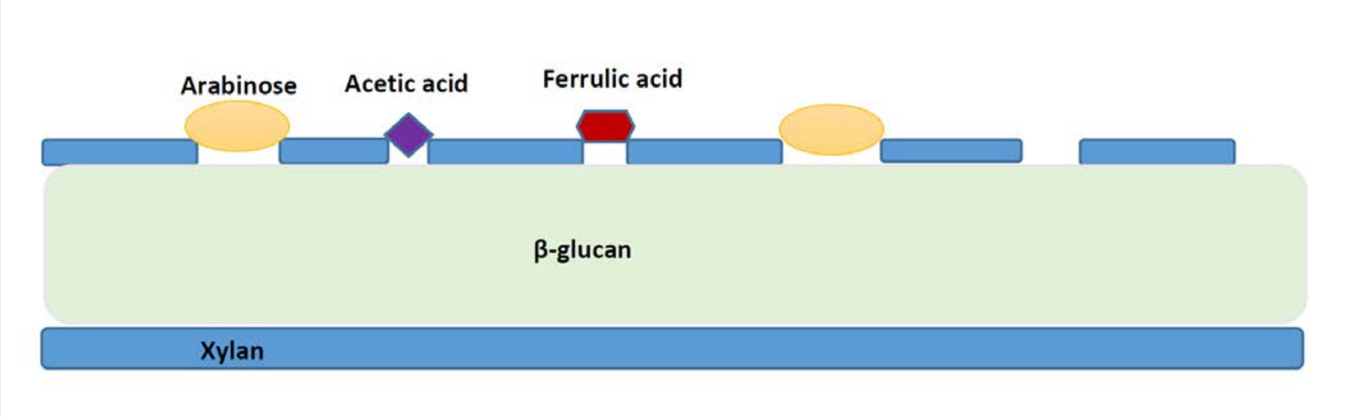
Agenda

- Where do these putative prebiotics come from?
- How to remove the term “putative” and confirm “prebiotic”?
- Looking ahead & what’s next?

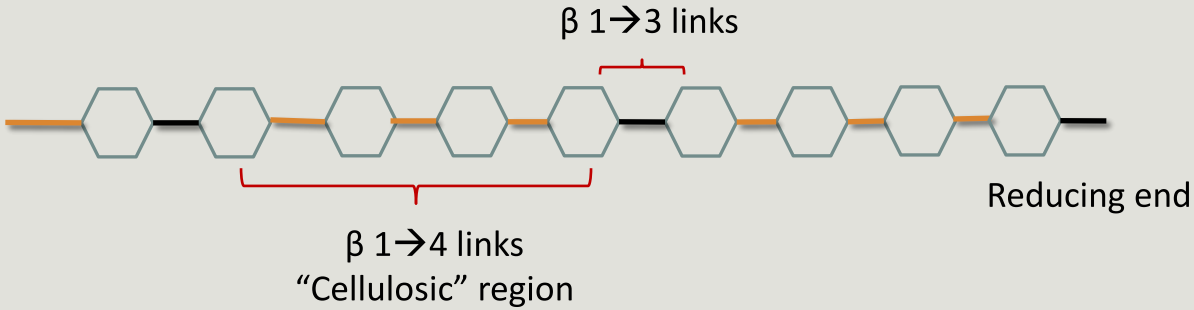
Origin of “putative” prebiotic compounds







Bamforth, C. W.; Kanauchi, M., A simple model for the cell wall of the starchy endosperm in barley. *J I Brewing* **2001**, *107*, 235-240.



Images adapted from:
Bamforth, C., *Scientific Principles of Malting and Brewing*. 2006. ; *C.W.B. UC-Davis FST 3 Course Notes*

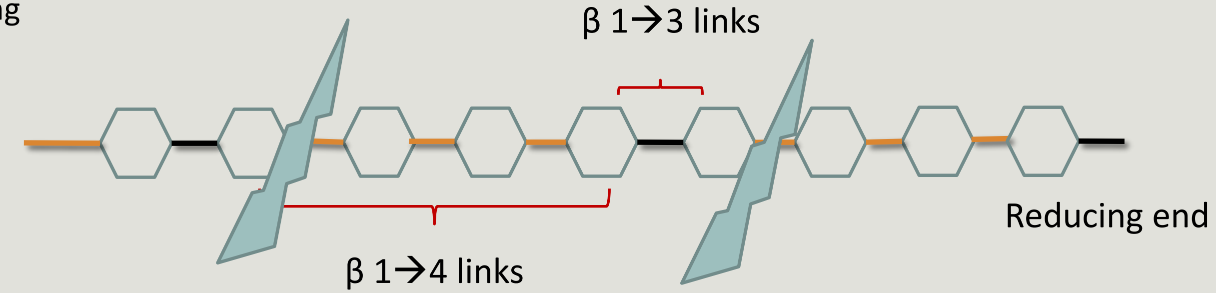
Breakdown of cell wall

Solubilization

- Cell wall structure attacked by solubilases – β-glucanases & pentosanases
- Carboxypeptidase acting on beta-glucan protein links

Hydrolysis

- Endo-barley-β-glucanase
  - Developed in carefully malted barley
  - Hydrolyzes β 1-4 bonds on reducing side of β1-3 bond.



Breakdown of the cell wall

Malting, & mashing goal: minimize β-glucan content

Inevitably get β-glucan (0.12 – 1.35 g / 355 mL)

- 1) High molecular weight → soluble fiber
  - Problematic in brew house
- 2) Low molecular weight → putative prebiotic
  - Low MW oligosaccharides predominates
  - Degree of Polymerization (DP) 3 and 4
  - Not fermented by brewing yeast, possibly make it to large intestine

May 2006, FDA includes barley

Bamforth, C.; Gambill, S., Fiber and Putative Prebiotics in Beer. *J. Am. Soc. Brew. Chem.* **2007,** *65* (2), 67-69.

Crucial Questions

1) DO THE OLIGOSACCHARIDES SURVIVE DIGESTION?

- Three phase digestion
  - Oral
  - Gastric
  - Intestinal
- Intense conditions (low pH, high enzymatic activity, continuous agitation and mixing)
- Complex process

2) ARE THE OLIGOSACCHARIDES ACTUALLY PREBIOTIC?

- Compounds must be **consumed or used** by microbiota in large intestine to *facilitate growth or beneficial action* of certain probiotic organisms – such as *Lactobacillus*.

… "non-digestible food ingredient that beneficially affects the host by selectively stimulating growth and/or activity of 1 or a limited number of bacteria in the colon."

Gibson, G. R.; Roberfroid, M. B., Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of nutrition* **1995,** *125* (6), 1401-12.

Phase 1

Model Hydrolysis of β-glucan

- Hydrolysis of high purity β-glucan by endo-barley-beta-glucanase (Lichenase).
  - Previous studies quantified 91-97% of breakdown products as DP3-4 beta-glucan oligosaccharides (BGOs).
- Monitored and analyzed via thin-layer chromatography
  - Easy separation of carbohydrates based on molecular weight
- Glucose removed via addition of brewing yeast.

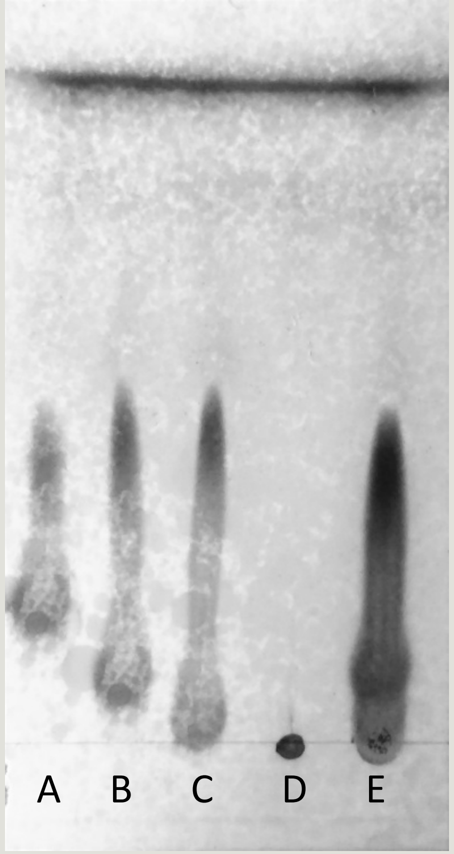


Figure 1. TLC of unhydrolyzed and hydrolyzed β-glucan. A 2% w/v solution of unhydrolyzed β-glucan (D) was enzymatically degraded using *endo*–1,3 (4)-β-Glucanase to yield a solution with newly generated, lower molecular weight β-glucan oligosaccharides (E). The hydrolysis products (E) were compared to DP 2 (A), DP 3 (B), and DP 4 (C) standard references (2.5µl, 5mg/ml).

Sims, I. M.; Ryan, J. L.; Kim, S. H., In vitro fermentation of prebiotic oligosaccharides by Bifidobacterium lactis HN019 and Lactobacillus spp. *Anaerobe* **2014**, *25*, 11-7

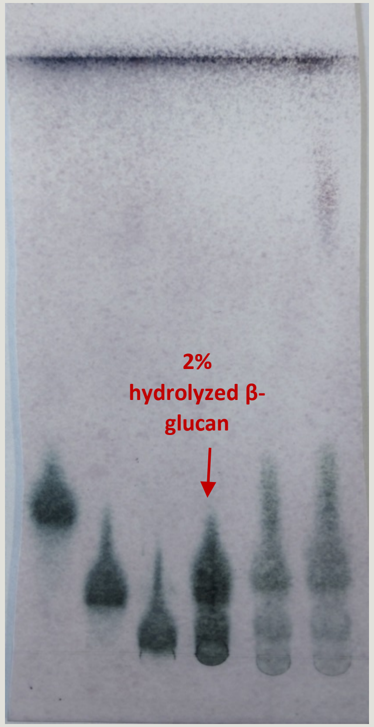
Phase 2

In vitro Digestive Model

- Collaboration with Dr. Gail Bornhorst at UC-Davis
- Simulated digestive process
- Three phases
  - Oral (30 s)
    - pH 7
    - Alpha-amylase
  - Gastric (2 hours)
    - pH 1.8
    - Pepsin
  - Intestinal (2 hours)
    - pH 6.5 – 7.0
    - Pancreatin

Digestive Phase	Protocol
Oral Phase	- Adjust saliva solution to pH 7 - Addition of α -amylase (1.18g/L) - Combine 5.0 mL substrate + 3.33 mL of enzymatically active saliva solution - Shake by hand for 30 seconds
Gastric Phase	- Adjust gastric juice to pH 1.8 - Addition of pepsin (1.0g/L) - Add 6.66 mL enzymatically active gastric solution to 8.33 mL of oral phase solution. - Incubate sample in 37˚C shaking water bath at 110 rpm for 40 minutes. - Check pH and readjust to 1.8 – 2.0 and continue to shake for additional 40 minutes. - 1 mL sample taken and neutralized using 0.1 M NaOH.
Intestinal Phase	- Adjust intestinal juice to pH 6.5 - Addition of pancreatin (2.4g/L) - Add 10 mL enzymatically active intestinal solution to gastric phase solution. - Check pH of solution and readjust to 6.5 as necessary - Incubate sample in 37˚C shaking water bath at 110 rpm for 2 hours - 1 mL sample taken and neutralized using 0.1M NaOH.

Digestion results



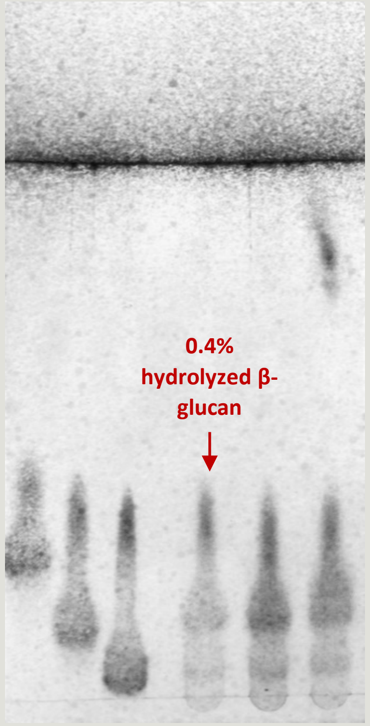


Figure 3. Thin layer chromatography plate of hydrolyzed and digested β-glucan oligosaccharides. A 2.5 μL aliquot of experimental samples and standards were applied to the Silica 60 glass backed TLC plate. The plate was run in a mixture of ethyl acetate, methanol, acetic acid and water in a ratio of 60:20:15:5, and the plates were stained with an anisaldehyde spray. From left to right: Column 1- cellobiose standard (5 mg/mL); Column 2- cellotriose standard (5 mg/ml); Column 3 - cellotetraose standard (5 mg/mL); Column 4– hydrolyzed β-glucan (2%, or 0.4% w/v respective TLC plates); Column 5 – post-gastric digestion of 2% w/v hydrolyzed β-glucan (final BGO concentration 0.67% w/v); Column 7 – post-intestinal digestion of 2% w/v hydrolyzed β-glucan (final BGO concentration 0.4% w/v). β-sdf

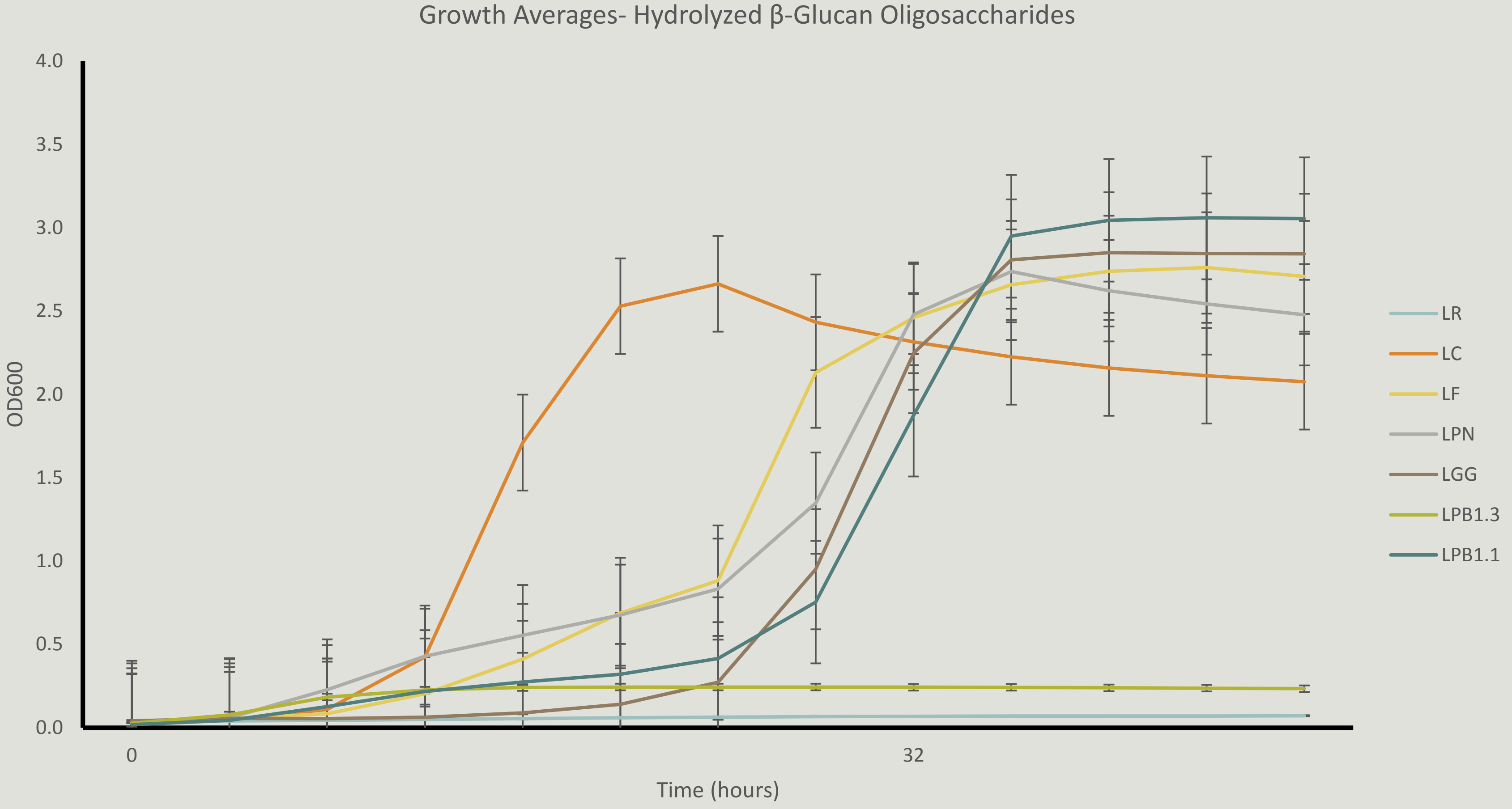
Phase 3

Microbiological Growth Studies

- Collaboration with Dr. Maria Marco at UC-Davis
- 7 strains of probiotic *Lactobacillus* (LAB) selected
- Microplate growth studies with different carbon sources
  - Overnight cultures in glucose
  - Examination of optical density values at 600 nm over 48h

Organism	Origin	Abbreviation Code
***Lactobacillus reuteri* ATCC 2327**	Human feces	LR
***Lactobacillus casei* BL23**	Cheese	LC
***Lactobacillus fermentum* B6**	Boza (fermented African beverage)	LF
***Lactobacillus plantarum* NCIMB8826**	Human saliva	LPN
***Lactobacillus rhamnosus* GG**	Intestinal tract	LGG
***Lactobacillus plantarum* B1.3**	Ethiopian injera dough (uncooked)	LB1.3
***Lactobacillus plantarum* B1.1**	Ethiopian injera dough (uncooked)	LB1.1

Microbiological Results



<u>5 out of 7</u> LAB strains capable of utilizing BGOs as energy source.

Organism	Maximum OD - Glucose	Maximum OD - BGO
LR	2.8	0
LC	3.0	2.75 *reached in < 24hrs
LF	3.0	2.75
LPN	3.2	2.75
LGG	3.2	2.8
LB1.3	3.0	0
LB1.1	3.4	3.0

In Summary

Major questions addressed:

1) Do the prebiotic BGOs survive the digestive process? ✓

2) Are the BGOs consumed and used by *Lactobacillus*? ✓

Incorporation of LAB into diet + moderate consumption of beer = improved gut health

Future Research

- Next steps…
  - Addressing digestion complexity
  - Animal trials
  - Clinical trials

Thank you.

- Charlie Bamforth & the Bamforth Lab
  - Joe Williams
  - Emma Kultgen
- Dr. Gail Bornhorst & the Bornhorst Lab
  - Yamile Mennah
- Dr. Maria Marco & the Marco Lab
  - Annabelle Yu
  - Irene Yim