Global Prebiotic Association Young Researcher Awards - Entry #348

Name

Bruna Paviani

Your Title

PhD Student

Your Affiliation

University of California, Davis

Email

bpaviani@ucdavis.edu

Phone

+15303027613

Please indicate which category you're applying for:

GPA Young Researcher Award for Applied Research (115 points possible)

Please provide a link to your published paper (if open access) or abstract:

https://www.sciencedirect.com/science/article/pii/S0141813023053710

Please provide a summary of your research(limit 250 words)

Bioactive oligosaccharides have been garnering global attention thanks to their potential to improve human health, especially in modulating gut microbiota via prebiotic activity, and have this far been most studied in human milk. Despite significant advancement in isolating oligosaccharides from mammalian milk, and obtaining a handful of structures via recombinant technologies, there has been limited progress in translating this science to practice. Therefore, the exploration of alternative (non-milk) sources for prebiotic oligosaccharides that can be matched to specific probiotic bacteria and the investigation of processing techniques for generating large quantities of oligosaccharides are important—yet challenging—tasks.

This work uses polysaccharide oxidative cleavage to generate oligosaccharides from beet pulp, an agroindustry by-product. A scalable membrane filtration approach was applied to purify the oligosaccharides for subsequent in vitro testing. An advanced mass spectrometry platform allowed discovering the oligosaccharide profile and their monosaccharide complexity, revealing 29 unique structures varying in size from 3 to 17 units. A final product containing ~40 g of oligosaccharide was obtained from 475 g of starting material (beet pulp pellets). Microbiological bioactivity assays indicated that the product obtained herein stimulated desirable commensal gut bacteria. This rapid, reproducible, and scalable method represents a breakthrough for generating potential

prebiotics from plant by-products at scale. The achievements of this work demonstrate the power of working with other disciplines and with industry to achieve important goals.

Please provide a summary of methods (limit 250 words)

Chemical depolymerization for oligosaccharides production using Fenton reaction was upscaled in a pilot plant using 6 kg of beet pulp paste as substrate. The reacted material was purified using a sequential membrane filtration (microfiltration and ultrafiltration to remove unreacted polysaccharides, nanofiltration to concentrate oligosaccharides, desalination to remove salts introduced by the reaction). Mass spectrometry tools were employed to assess the oligosaccharides structure:
• Beet pulp paste and enriched FITDOG oligosaccharides were analyzed with an Agilent 6520 Accurate-Mass quadrupole time-of-flight (Q-TOF) liquid chromatography-mass spectrometry (LC/MS) system equipped with a microfluidic chip cube interface to asses degree of polymerization, number of unique structures and class of monosaccharides present in the oligosaccharides (hexoses, pentoses, hexuronic acids).
• The monosaccharide composition of the oligosaccharides was determined by acid hydrolysis with 4 M TFA, derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP) and samples were analyzed on an Agilent 6495 QqQ MS coupled to an Agilent 1290 Infinity II UHPLC.
Bioactivity investigation was assessed by culturing select probiotic species (Bifidobacterium catenulatum JCM15439, Bifidobacterium bifidum SC555, Lactiplantibacillus plantarum ATCC 8014, and Limnosilactobacillus reuteri DSM 20016) tested for the ability to grow in the presence of enriched FITDOG oligosaccharides as the sole carbon source.

Please provide a summary of your results (limit 250 words)

This work was highly interdisciplinary, involving analytical chemists, food scientists and microbiologists as well and an entire R&D team from the industry sponsor (Marc Inc.). The Fenton oxidative cleavage was successfully performed for the first time at a pilot scale, producing 40 g of enriched oligosaccharides from beet pulp pellets, a by-product of the sugar industry. Overall, 29 unique oligosaccharides structures were identified via LC-MS. The obtained oligosaccharides presented a degree of polymerization between 3 and 17 units and building blocks made of hexoses, pentoses, and hexuronic acids, a more diverse and abundant composition than the naturally occurring oligosaccharides in beet pulp. Widely used green techniques such as membrane filtration and desalination, which are further scalable at the industrial level, were optimized to purify the generated oligosaccharides and eliminate simple sugars and salts for subsequent bioactivity investigation. Beet pulp oligosaccharides revealed selective bifidogenic activity and moderate effect on lactobacilli, demonstrating potential as a novel prebiotic to be further investigated. It is important to remember that oligosaccharides are not all the same in terms of efficacy. Indeed, the prebiotic properties of oligosaccharides highly depend on their structural conformation. From the industrial point of view, this method and the associate library of oligosaccharide structures has the potential to be adapted for rapid identification of oligosaccharides in situations where fast results are desirable, such as to facilitate oligosaccharides extraction and quality control at the industrial level after performing the Fenton reaction.

Please provide a statement about what, in your opinion, makes this paper outstanding and why it fits into the grant category you selected. (limit 250 words)

This work proposes an innovative technology based on polysaccharide oxidative cleavage and multi-stage membrane purification to produce potential prebiotic oligosaccharides from renewable sources, allowing a higher-value added to a current low-value material, that can also be applied to other high-fiber agrifood-waste. It also provides critical information to evidence the prebiotic potential of the newly generated oligosaccharides on

the growth promotion ability of representative probiotic strains of bifidobacteria and lactobacilli. Having this toolset in place will prompt other scientists to purse this research and further generate bioactive oligosaccharides in more agricultural waste streams to improve human health. As a matter of fact, encouraged by our successful proof-of-concept, a start-up company (https://one.bio/) was recently spun-off our university to take this science into commercial reality.

Our process produces safe, probiotic-fermentable oligosaccharides. According to the FDA, the genus Bifidobacterium is Generally Recognized as Safe (GRAS), and many probiotics contain one or more Bifidobacterium species. The mechanisms by which probiotic administration benefits humans are multifactorial, but the main one is via short-chain fatty acids and lactic acid production after consuming prebiotics. Identifying key carbohydrate structures that support the growth of probiotics will promote research dissecting mechanisms, identifying new strains, and carbohydrates fermentable only by desirable species. There is a pressing need to identify additional technological platforms (such as the one presented here) that can produce a wide diversity of oligosaccharides at scale to enable rigorous testing and hypothesis-driven research of benefits. Importantly, the development of plant-based platforms has larger implications for producing a much wider set of bioactive oligosaccharides to investigate their putative health benefits than currently available via chemo-enzymatic/recombinant methods.

By typing your full name below and completing this application, you verify that you are the first author of this research and that this paper is original research.

Bruna Paviani