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The water-soluble non-starch polysaccharides from bananas display immunomodulatory properties on cultured macrophages

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ABSTRACT

Some diet components, such as certain indigestible polysaccharides from edible plants, may interact with the gut-associated lymphoid tissue and improve the host immune response to pathogens. The non-starch polysaccharides (NSP) from bananas are non-digestible carbohydrates that resemble some immunomodulatory polysaccharides occurring in the cell wall of cereals. Based on this similarity, the effects of the water-soluble NSP from two banana cultivars (Nanicão and Thap Maeo) on the phagocytic activity, nitric oxide (NO) and cytokines produced by cultured macrophages were investigated. An investigation into the monosaccharide composition and the oligomers released by enzymatic hydrolysis of the ultra-filtered fraction above 50 kDa and the heat-treated fraction of water-soluble NSP from both cultivars revealed they are mostly composed of mannan and galacturonans (homogalacturonan, xylogalacturonan and rhamnogalacturonan). The NSP tested were able to activate the macrophages, but the effects on the phagocytic activity and the release of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and nitric oxide production were dependent on the polysaccharide concentration and the cultivar origin. Despite some specific differences, the NSP from Nanicão and Thap Maeo banana fruits may be considered prospective food immunomodulators, which contribute to the promotion of a more responsive immune system.

1. Introduction

Some diet components, as is the case for certain indigestible polysaccharides, may activate the host immune system through direct or indirect interaction with gut-associated lymphoid tissue (GALT) (Chu & Mazmanian, 2013; Sellers & Morton, 2014; Wismar, Brix, Frøkiaer, & Laerke, 2010). These biological response modifiers (BRM) from among edible fungi, algae and plants are able to interact with various types of immune cells from the mucosal-associated lymphoid tissue (MALT) through direct receptor activation or secondary activation by soluble components of the immune system, such as chemokines and cytokines (Boh, 2013; Chandrashekar, Prashanth, & Venkatesh, 2011; Ramberg, Nelson, & Sinnott, 2010; Yu, Nie, Li, Zheng, et al., 2013). Polysaccharides have the potential to improve the capacity of some defense cells to recognize and destroy possible pathogens with more efficiency, or even to prime macrophages, the first-line defense cells, and boost their phagocytic capacity (Pereira et al., 2000; Schepetkin & Quinn, 2006; Tan, Li, Lai, & Zhang, 2013; Yu et al., 2013). It is well known that beta-glucan and type II arabinogalactan and other polysaccharides activate monocytes, macrophages and dendritic cells. In this regard, several studies have shown that polysaccharides derived from edible fruits are BRM (Burana-Osot, Pattanapanyasat, Soonthornchareonnon, Sukapirom, & Toida, 2010; Cherng, Chiang, & Chiang, 2007; da Silva, Tostes, & Parente, 2000; Ramberg et al., 2010; Schepetkin & Quinn, 2006). For example, xyloglucans extracted from the cell wall of many commercial fruits (kiwi, peach, avocado, apple, pineapple and tangerine) and their oligosaccharides, inhibit the growth of the human tumor COLO 201 cell line, through activation of immune cells and cytokine release (Kato, Uchida, Ito, & Mitsuishi, 2001).

Bananas, one of the most consumed fruits worldwide, have a significant amount of carbohydrates. Starch and the soluble sugars sucrose, glucose and fructose are digestible carbohydrates found abundantly in the pulp of banana; their relative proportions vary depending on the cultivar. The non-digestible carbohydrates from banana are represented by non-starch polysaccharides (NSP), mainly pectin and hemicellulose,

Abbreviations: BRM, Biological Response Modifier;; GALT, Gut Associated Lymphoid Tissue; HTP, Heat-Treated polysaccharide; IFN-γ, Interferon gamma; IL-6, Interleukin-6; MALT, Mucosal Associated Lymphoid Tissue; NO, Nitric Oxide; NSP, Non-Starch Polysaccharides; TNF-α, Tumor Necrosis Factor Alpha; UFP, Ultra-Filtered Polysaccharides; WSP, Water Soluble Polysaccharides.

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which are usually associated with the soluble and insoluble fractions of dietary fiber, respectively (Cordenunsi, Shiga, & Lajolo, 2008). These components of the dietary fiber are polysaccharide chains with glycoside linkages β(1 → 3), β(1 → 4), β(1 → 6), which are not hydrolyzed by the endogenous enzymes of the human gastrointestinal tract. According to Cordenunsi et al. (Cordenunsi et al., 2008), NSP from banana seems to be composed of galacturonans and arabinoxylans, which resemble the composition of the primary cell wall of cereals. This similarity could be explained by the phylogenetic proximity between bananas and cereals since the former are members of the *Zingiberales* order, and the second are members of *Poales*, both from the commelinids clade of monocots. The cell wall of grasses and cereals such as oats are characterized by a rich content of arabinoxylan, arabinogalactan and uronic acids like homogalacturonans and type II rhamnogalacturonans of the pectins. These classes of polysaccharides, existing in fungi, algae and other higher plants have been studied for their immunomodulating potential.

Based on the above information it is plausible to consider that water-soluble NSP present in banana fruit may also have immunomodulatory properties. Also, as Cordenunsi et al. (Cordenunsi et al., 2008) reported, the banana cultivars Nanicão and Mysore had similar NSP content, but differed substantially in CDTA (Ca-linked pectin) and NaOH-4M (hemicellulose) soluble fractions. Additionally, the Nanicão had more calcium-linked pectin while Mysore was richer in hemicellulose-like polysaccharides. Thus, there could be differences in the immunomodulatory potential of NSP from different banana cultivars. In order to investigate this question, the water-soluble NSP from the banana cultivars Nanicão and Thap Maeo, a Mysore clone, were assayed in vitro with cultured macrophages, and the effects on the phagocytic activity, nitric oxide and cytokine production were investigated.

2. Materials and methods

2.1. Materials

Fully ripe bananas (*Musa* spp.) of cultivars Nanicão (Group AAA) and Thap Maeo (Group AAB) with a peel color index of 6 were obtained in a wholesale market. The ripening stage was confirmed by the quantitation of starch and soluble sugars in the fruit pulp, as previously reported (Cordenunsi et al., 2008; Shiga et al., 2011). The fruits were peeled, sliced, and the pulp was frozen in liquid nitrogen and ground with a mortar and pestle and stored at −80 °C. The RAW 264.7 macrophages were mycoplasma free and obtained at the 13th subculture. The solvents used for chromatography were HPLC grade, and all other chemicals (reagents and solvents) were of analytical grade, culture media grade or molecular biology grade, as appropriate.

2.2. Extraction of WSP

Five grams of frozen banana pulp of each cultivar was ground in a mortar and pestle in the presence of liquid nitrogen and was added to 250 ml methanol-chloroform (1:1 v/v) at 70 °C for an hour under reflux in order to remove protein, lipid, pigments and free phenolic compounds besides enzymes inactivation. Sevag method was employed to remove proteins, but the previous step seems like removing all free protein from the sample, as observed in dosage using Bradford methodology (data not shown). The suspension was centrifuged (6500 ×*g*, 10 min, 4 °C) and the precipitate was washed three times with acetone. After drying at 40 °C, the material was ground and extracted with 10 ml of deionized water for 1 h in an ultrasonic bath, with stirring at each 10 min interval. The suspension was centrifuged (10,000 ×*g*, 10 min, 4 °C) and the supernatant was precipitated overnight at 4 °C by adding absolute ethanol to the final concentration of 80%. The solution was centrifuged as above, and the precipitate was washed three times with 80% ethanol to remove mono- and oligosaccharides. The precipitate was resuspended in 30 ml of deionized water and dialyzed for 72 h at 4 °C using a 3500 Da cut-off membrane. After centrifugation (10,000 ×*g*, 10 min, 4 °C) of the retentate, the supernatant containing the water-soluble polysaccharides (WSP) was obtained and freeze-dried for storage. The absence of starch in the sample was confirmed by using the Megazyme Starch Assay to test for the presence of residual starch.

2.3. Purification of WSP

A 50 ml aliquot of a 0.15% solution of WSP was submitted to tangential ultra-filtration in a 50 kDa Pellicon XL cassette (Millipore, Darmstadt, Germany). Filtration proceeded for 3 h under a 35 ml·min^{-1} flow and the filter-retained fraction of polysaccharides above 50 kDa, was collected and submitted to chromatographic separation on a 1 ml HiTrap Q Sepharose FF column (GE Healthcare Life Sciences, Pittsburg, PA). The column was equilibrated with successive washes with 2 M, 1 M NaCl and deionized water, for 10 min each; at a flow rate of 0.825 ml·min^{-1}. The equilibrated column was loaded with a 1 ml aliquot of a 15 mg·ml^{-1} solution of ultra-filtered polysaccharides and washed with ten volumes of deionized water. The polysaccharides bound to the resin were released with successive washes with 4.125 ml of 0.05, 0.1, 0.2, 0.5 and 1 M NaCl, for 5 min each, and the total carbohydrate content was monitored using the Phenol-sulfuric Dubois method adapted for use in a microplate (Masuko et al., 2005). The eluted fractions with 0.5 and 1 M NaCl of purified ultra-filtered polysaccharides above 50 kDa (UFP) were pooled, dialyzed against deionized water, freeze-dried.

2.4. Thermal treatment of WSP

Ten milligrams of the freeze-dried UFP preparation was solubilized in 10 ml of deionized water and heated at 121 °C for 15 min. The solution of polysaccharides was freeze-dried for storage and considered the heat-treated polysaccharides (HTP).

2.5. Monosaccharide analysis

The monosaccharide composition of WSP was analyzed according to Shiga et al. (2011). One milligram of polysaccharide preparation was hydrolyzed with 2 M TFA at 120 °C for 1 h and the supernatant was recovered by centrifugation at 2000 ×*g* for 5 min. One milliliter of tertbutyl alcohol was added to the supernatant, and the mixture was dried under N_2 stream. The residue was solubilized in water and analyzed by HPLC-PAD using a DX 500 system (Dionex, Sunnyvalle, CA) with a CarboPac PA10 column (Dionex). Neutral sugars were separated in water with postcolumn adjustment with 300 mM NaOH for detection, while the uronic acids were separated in 150 mM NaOH and 0–220 mM sodium acetate gradient. Standards of arabinose, fucose, galactose, glucose, mannose, rhamnose, xylose, galacturonic and glucuronic acid were considered in the identification and calculation of the percentage of monosaccharides.

2.6. Polysaccharides enzymatic hydrolysis

Suspensions containing 10 mg·ml^{-1} of UFP or HTP were prepared using sodium acetate buffer pH 5.5, 4.5 and 4.0, glycine buffer pH 8.8 and potassium phosphate buffer pH 7.0, all at 100 mM. The suspensions were incubated separately using, respectively, 5 μL (5 U) of xyloglucanase from *Paenibacillus* sp. (E.C. 3.2.1.151; Megazyme International Ireland; Wicklow, Ireland); 3 μl (5 U) of β-xylanase from *Trichoderma viride* (E.C. 3.2.1.8; E-XYTR1; Megazyme); endopolygalacturonase from *Aspergillus niger* (E-PGALS; E. C. 3.2.1.15; Megazyme); 10 μl (3.9 U) of *endo*-(1,4)-β-mannanase from *Bacillus* sp. (E.C. 3.2.1.78; E-BMABS; Megazyme); 5 μl (5 U) of *endo*-/*exo*-arabinanase of *Cellvibrio japonicus* (E.C. 3.2.1.99; E-ARBACJ; Megazyme). All mixtures were incubated at 40 °C for 2 h and filtered through 40 μm filter. The hydrolysates were analyzed using a PA1 pellicular anion-exchange analytical (Dionex, 250 × 4 mm) column with its respective