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Effects of whey peptide extract on the growth of probiotics and gut microbiota

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ABSTRACT

Whey peptide extract with molecular weight below 1 kDa was investigated in microplate assay, and viable cells, as well as metabolic activity were determined to evaluate augmented growth of probiotic bacteria (*Lactobacillus acidophilus* and *Bifidobacterium animalis*). Results illustrated that whey peptide extract 1% (w/v) has the capacity to stimulate the proliferation of both probiotic bacteria tested, further supported by the faster generation of metabolic products. The effect of whey peptide extract on the modulation of gut microbiota was also examined in Wistar rats fed either with a standard or a high-fat diet, assessed via 16S ribosomal RNA expression of gut microbiota by quantitative PCR. Relative abundance of *Lactobacillus* spp., *Bifidobacterium* spp. and Bacteroidetes was significantly increased by whey peptide extract in rats fed with a standard diet. These results highlight an additional unexploited positive effect of whey peptide extract on gut microbiota modulation.

1. Introduction

As a major waste of cheese industry, whey has been given increasing importance not only due to its nutritious value but also due to concern of environmental pollution (Pintado, Pintado, & Malcata, 1999; Tavares & Malcata, 2013). Whey contains highly valuable soluble milk components, particularly soluble proteins (0.6–0.8%, w/v) (Barth & Behnke, 1997; Walzem, Dillard, & German, 2002), and the role of these soluble proteins has been increasingly emphasised due to the discoveries on their bioactive properties (Sinha, Radha, Prakash, & Kaul, 2007; Urista, Fernandez, Rodriguez, Cuenca, & Jurado, 2011).

By definition, bioactive peptides are certain protein fragments exhibiting positive health impacts (Kitts & Weiler, 2003). Although bioactive whey peptides are less common than those of casein, there have been some studies illustrating their diverse biological functions (Lòpez-Expòsito & Recio, 2006; Marques et al., 2015; Ortiz-Chao et al., 2009; van der Kraan et al., 2005).

On the other hand, consumption of prebiotics (Grootaert et al., 2009) to modulate microbiota, such as stimulating proliferation of *Bifidobacterium* and *Lactobacillus* genera (Tuohy,

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Rouzaud, Bruck, & Gibson, 2005), which brings about the healthy impacts (Wang, 2009), has been also increasingly focused. However, most substances identified as prebiotics or candidates are currently non-digestible oligosaccharides (Crittenden & Playne, 1996), i.e., the potential of proteins/peptides as prebiotics, leading to growth promoting effect and improvement of metabolism, has not yet been fully explored.

Various microorganisms exist in the human intestinal tract and bacteria predominate among them. Advanced methods, for instance 16S ribosomal RNA (rRNA) investigations and direct sequencing of faecal microbiota, have facilitated findings on the relationship between gut microbial communities and host metabolism. More than 90% of those findings are associated with Firmicutes and Bacteroidetes (Tremaroli & Backhed, 2012; Vrieze et al., 2010), and host obesity was found to alter their colonisation (Furet et al., 2010; Ley et al., 2005). On the other hand, Actinobacteria (ex. *Bifidobacterium*) and Proteobacteria (ex. *Helicobacter* and *Escherichia*) also constitute a great number among gut bacteria (Eckburg et al., 2005; Ley et al., 2005).

In the current study, a new whey peptide (<1 kDa) extract (WPE) obtained from whey hydrolysed by *Cynara cardunculus* was evaluated to determine its impacts on the modulation of gut beneficial bacteria. Initial screening and conventional microbiological analysis of the single strains, *Bifidobacterium animalis* subsp. *lactis* (strain Bb 12) and *Lactobacillus acidophilus* Ki were undertaken to examine *in vitro* direct effects on augmented growth of specific bacteria, allowing a more complete understanding of their probable mechanism. Additionally, animal experiments were also conducted to examine whether the WPE can modify the composition of colonic microbiota in two different dietary patterns (standard and high-fat diet), by quantifying 16S rRNA gene expressions of certain gut microbe communities through real-time PCR.

2. Materials and methods

2.1. Materials and microorganisms

Fructooligosaccharide (FOS) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Chemical standards utilised in HPLC included 98% sulphuric acid (Merck KGaA, Darmstadt, Germany), glacial acetic acid (Fisher Scientific, Leics, UK), L-(+)-Lactic acid and glucose (Sigma-Aldrich). Microbial culture media (MRS broth and MRS agar) were purchased from Biokar Diagnostics (Beauvais, France). *Lactobacillus acidophilus* Ki, previously isolated from fermented milk, was obtained from CSK Food Enrichment (Leeuwarden, The Netherlands) as ultrafrozen concentrates, and *Bifidobacterium animalis* Bb12 was obtained from Christian Hansen (Hørsholm, Denmark) as lyophilised culture.

2.2. Preparation of WPE

Whey extracts were obtained and processed according to Tavares et al. (2012) with some modifications. Mixture of 80% cow, 10% goat and 10% ewe whey was kindly provided by Saloio (Torres Vedras, Portugal). Hydrolysis of whey was performed using aqueous extract of *Cynara cardunculus* (Formulab, Maia, Portugal) (Tavares et al., 2011) at an enzyme/substrate ratio of 3.0% (v/v), incubated for 3 h at pH 5.2 and 55 °C. Besides, further processes were done using a 1 kDa cut-off membrane to generate the filtrates, constituting the peptides with MW <1 kDa. The WPE with MW <1 kDa was further used in the following experiments to assess its probiotic growth enhancing properties.

2.3. Screening of bacterial growth via microplate assay

To evaluate the role of the WPE as prebiotic (assuming the enhancing properties upon probiotic bacteria), the microplate assay was conducted with variable media-pure MRS broth, MRS broth with 2% (w/v) FOS or different concentrations of the whey extract (1.0 and 2.0%). MRS broth was autoclaved at 121 °C for 20 min and subsequently supplemented with filter-sterilised FOS and 0.5 g/L of L-cysteine (for the growth of *B. animalis* Bb12).

Owing to the lower solubility of whey extract, filter sterilisation could not be used, but the broth with the extract had to be sterilised at 80 °C for 30 min. In order to control the sterility of this medium, a control without inoculum was always included to prove the absence of growth. Each type of medium was then inoculated at 2% (v/v) with each of aforementioned probiotic bacteria. One aliquot of 250 μL was transferred to a 96-well microplate (Thermo Fischer Scientific, Denmark) in triplicate and the wells were covered with 50 μL autoclave-sterilised liquid paraffin (Merck, Germany) to avoid the presence of oxygen. Incubation occurred at 37 °C for a period of 48 h under non-controlled pH conditions, and cellular growth was monitored by measuring the OD of the cultures at 660 nm at intervals of 60 min using microplate reader (FLUOstar OPTIMA, BMG LABTECH GmbH, Ortenberg, Germany).

2.4. Evaluation of fermentable activity via determination of viable cells and metabolic activity

Effect of peptide extracts on the growth of probiotics was evaluated as the following. After completely dissolving, the medium with the extract was specially sterilised at 80 °C for 30 min as previously explained. *L. acidophilus* Ki and *B. animalis* Bb12 were inoculated at 2% (v/v) into basal media MRS, with filter-sterilised FOS 2% (medium used as positive control since FOS is one of the most used prebiotics) and WPE (1% and 2% w/v) for 24 h and 48 h, respectively. The culture was then transferred to 15-mL sterile tubes and incubated at 37 °C for 24 h. All the conditions were sampled at 8 or 9 time points (0, 2, 4, 6, 8, 10, 12 and 24 h for *L. acidophilus* Ki and 48 h additionally for *B. animalis* Bb12). At each sampling point, inoculated medium (100 μL) was decimally diluted in salt peptone (1 g/L) (Sigma-Aldrich), and plated in duplicate on two types of media: MRS agar for *L. acidophilus* Ki and MRS agar added with 0.5% L-cysteine for *B. animalis* Bb12. Inoculation, incubation and enumeration were conducted as described previously by Miles, Misra, and Irwin (1938).

Of each triplicated sample 500 μL was diluted in 500 μL of sulphuric acid 13 mM in a single run and the concentration was obtained based on calibration curves previously prepared with appropriate chromatographic standards. HPLC analysis was conducted by referring to Zeppa, Conterno, and Gerbi (2001) in order to assess the metabolic activity of each probiotic in various medium. The HPLC system consists of a LaChrom L-7100 pump (Merck-Hitachi, Fullerton CA, USA); an