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Dairy propionibacteria prevent the proliferative effect of plant lectins on SW480 cells and protect the metabolic activity of the intestinal microbiota *in vitro*

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ABSTRACT

Plant lectins are specific carbohydrate-binding proteins that are widespread in legumes such as beans and pulses, seeds, cereals, and many plants used as farm feeds. They are highly resistant to cooking and digestion, reaching the intestinal lumen and/or blood circulation with biological activity. Since many legume lectins trigger harmful local and systemic reactions after their binding to the mucosal surface, these molecules are generally considered anti-nutritive and/or toxic substances. In the gut, specific cell receptors and bacteria may interact with these dietary components, leading to changes in intestinal physiology. It has been proposed that probiotic microorganisms with suitable surface glycosidic moieties could bind to dietary lectins, favoring their elimination from the intestinal lumen or inhibiting their interaction with epithelial cells. In this work, we assessed *in vitro* the effects of two representative plant lectins, concanavalin A (Con A) and jacalin (AIL) on the proliferation of SW480 colonic adenocarcinoma cells and metabolic activity of colonic microbiota in the absence or presence of *Propionibacterium acidipropionici* CRL 1198. Both lectins induced proliferation of colonic cells in a dose-dependent manner, whereas ConA inhibited fermentative activities of colonic microbiota. Pre-incubation of propionibacteria with lectins prevented these effects, which could be ascribed to the binding of lectins by bacterial cells since *P. acidipropionici* CRL 1198 was unable to metabolize these proteins, and its adhesion to colonic cells was reduced after reaction with Con A or AIL. The results suggest that consumption of propionibacteria at the same time as lectins could reduce the incidence of lectin-induced alterations in the gut and may be a tool to protect intestinal physiology.

1. Introduction

Dietary components influence a host's homeostasis in multiple ways, and a direct correlation between food and health is universally accepted. In addition to nutrients, many anti-nutritional and/or potentially toxic compounds are ingested daily in the diet by humans and animals. These substances could be endogenous components of food or exogenous contaminants. Among the endogenous components, plant lectins are specific carbohydrate-binding (glyco)proteins that are widespread in legumes such as beans and pulses, seeds, cereal grains, and other plants of the Leguminosae and Gramineae families used as farm feeds [1]. Although the amounts of lectins in foodstuffs can vary considerably, dietary intake by humans and animals can be significant. Their main role in plants is to act as toxins or defense proteins that protect the host from phytopathogenic microorganisms, insects and nematodes and other predators such as plant-eating animals and humans [2]. As a rule, these compounds show varying amount of resistance to inactivation by cooking and by digestive processes, reaching the intestinal lumen and/or blood circulation unaltered of with some biological activity. Their effects in the host are diverse depending on their origin and concentration, and both beneficial and deleterious effects have been reported for plant lectins [3–6]. As an example, some studies have shown that short-term exposure to lectins induce structural and functional maturation of the gut to better prepare farm animals to digest and absorb post-weaning feed and as a therapy for individuals having defective growth of the mucosa during total parenteral nutrition [7,8]. Lectins are also recognized as invaluable tools in glycobiology immunomodulation and cancer research [9].

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However, many authors agree that binding of legume lectins to membrane glycosyl groups of the cells lining the digestive tract triggers a series of harmful local and systemic reactions that allow placing these molecules as antinutritive and/or toxic substances. Their toxicity could be acute or chronic with morphological and physiological changes in the intestinal mucosa, such as shortening and shedding of microvilli and inhibition of digestive enzymes that can lead to a reduced absorptive function and less efficient feed conversion [10–13]. Lectins also locally affect the turnover of gut epithelial cells, stimulate shifts in the bacterial flora and modulate the immune state of the digestive tract [12,14,15]. Systemically, they can disrupt lipid, carbohydrate and protein metabolism [11,12,16]; promote enlargement and/or atrophy of internal organs and tissues [12,17]; and alter the hormonal and immunological status [16]. It has been reported that many lectins, such as Concanavalin A (ConA) and peanut agglutinin (PNA), have mitogenic properties in hematologic cells, increase cellular proliferation and turnover of intestinal epithelium in rats and affect cell growth of colorectal cancer cell lines in a dose-dependent manner [8,10,18]. Because of the damage they cause to epithelial cells and their hypertrophic effects, some lectins have been considered as tumour promoters [8,18–22]. In contrast, antineoplastic properties based on regulation of apoptosis and autophagy have been reported for other lectins in recent years [6]. Consequently, the role in host health of each dietary lectin consumed in foods should be carefully evaluated.

In recent decades, studies on the probiotic properties of microorganisms suitable for the development of functional foods have significantly increased, as has the interest of both industry and consumers on these healthy products. In addition to the well-known properties of probiotics (such as immunomodulation, pathogens inhibition and regulation of metabolism), it has been demonstrated that probiotics can remove and metabolize toxins, carcinogens and anti-nutritional compounds from food and/or the gastrointestinal tract and even induce changes in host physiology that lead to a decrease in the deleterious effects caused by these substances on the consumer's health [23–25].

Classical propionibacteria are microorganisms of interest due to their technological properties as dairy starters, biological producers of propionic acid and bioactive compounds (vitamins B and K, conjugated linoleic acid, exopolysaccharides, trehalose, and bacteriocins) as well as for their probiotic properties [26,27]. Both *in vitro* and *in vivo* studies have demonstrated that propionibacteria are able to modulate in a favorable manner gut physiology, microbiota composition and immunity [27]. Accordingly, *P. freudenreichii* and *P. acidipropionici*, have been included in the list of agents recommended for Qualified Presumption of Safety (QPS) by the European Food Safety Authority [28].

Propionibacterium acidipropionici CRL 1198, is an Argentinean dairy strain with vast evidence of probiotic potential [12,29–36]. In previous studies, we observed that this strain has the ability to bind and remove some dietary lectins decreasing, in turn, their toxic effects on normal colonic cells [34]. In addition, consumption of propionibacteria at the same time as ConA, reduced the incidence of structural and physiological lectin-induced intestinal alterations in Balb/c mice suggesting that this strain could be considered as a tool to protect the intestinal mucosa from some anti-nutritional components commonly present in food [12].

The aim of the present study was to assess the effects of two representative lectins (ConA and AIL) on SW480 cell line proliferation and the metabolic activity of colonic microbiota and the ability of *P. acidipropionici* CRL 1198 to modify their bioavailability in the colon.

2. Materials and methods

2.1. Lectins

The following lectins, selected for their primary specificity for different carbohydrates were used in this study: ConA (concanavalin A from *Canavalia ensiformis)* with affinity to glucose/mannose; AIL (jacalin from *Artocarpus integrifolia*) and SBA (soybean agglutinin from *Glycine max*), which binds to galactose/β-1,3-N-acetylgalactosamine; WGA (wheat germ agglutinin from *Triticum vulgaris),* which binds to sialic acid/N-acetylglucosamine and UEA (Ulex europaeus agglutinin from *Ulex europaeus*), which binds to L-fucose. All chemicals were provided by Sigma Chemical Co, St Louis, MO, USA.

2.2. Microorganism and growth conditions

Propionibacterium acidipropionici CRL 1198 was isolated and characterized for probiotic properties in Laboratorio de Ecofisiología Tecnológica of CERELA (CRL: Centro de Referencia para Lactobacilos, CERELA, Tucumán, Argentina). The strain stored at −20 °C in 10% (w/v) reconstituted skim milk containing 5 g/L yeast extract was propagated in LAPTg broth (15 g/L peptone, 10 g/L tryptone, 10 g/L glucose, 10 g/L yeast extract and 1 g/L Tween 80, pH 6.8) at 37 °C and subcultured at least twice in this medium every 24 h. For experimental use, a 48-h culture of *P. acidipropionici* CRL 1198 (5×10^9 CFU mL^{-1}) was harvested by centrifugation (3000 × g, 10 min, 4 °C), washed with pre-reduced sterile saline solution (9 g/L NaCl) and suspended in one-tenth of the original volume in the same solution (5×10^{10} CFU mL^{-1}). This suspension was used to inoculate, when necessary, growth media and mice intestinal homogenates.

2.3. Human colon cancer cell line

The human SW480 colon adenocarcinoma cell line (kindly provided by Dr. Joan Villena, Universidad de Valparaíso, Chile) was used as an *in vitro* model. Cells were routinely grown in D-MEM/F12 medium (Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated (30 min at 56 °C) fetal bovine serum and a mixture of antibiotics (50 U/mL penicillin, 50 μg/mL streptomycin, and 1.25 μg/mL amphotericin B) at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Monolayers of SW480 cells were obtained in 24-well tissue-culture plates by inoculating 2×10^4 viable cells per well in 1 mL culture medium. Cells were incubated until they reached a confluent state (approximately 10^7 cells/ml) with a change of culture medium every 2 days [35].

2.4. Effect of lectins and propionibacteria on proliferation of colon cancer cell line

For this assay, SW480 cells were seeded into 96-well plates (Corning Inc., Corning, NY) at a concentration of 1×10^4 cells/mL per well. Lectins were dissolved in D-MEM/F12 and then added into the plates in order to obtain final concentrations of 50, 100, 250 and 500 μg/mL lectins per well (three wells per concentration in two different assays). A 48-h-grown culture of propionibacteria (stationary phase of growth) was centrifuged and the supernatant was discarded. The pellet was washed with PBS and resuspended in D-MEM/F12 at a concentration of approximately 1×10^8 CFU/mL. One milliliter of propionibacterial suspensions was preincubated with ConA and AIL in the concentrations used for adenocarcinoma cells (50, 100, 250 and 500 μg/mL) for 60 min at 25 °C.Then, mixtures were centrifuged (10000 × g, 10 min, 4 °C) and supernatants were added to SW480 monolayers. Bacterial cells obtained from each

sample were stored at 4 °C to assess the effect of lectins on the ability of propionibacteria to adhere to colonic cells. The assay was also carried out by including solutions of 50 mmol/L D-mannose and N-acetyl-D-galactosamine in the reaction mixtures containing 100 μg/mL lectins. The plates were incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C for 48 h. The assay was finished by fixation *in situ* of intestinal cells with 20 μL of 50% cold trichloroacetic acid (TCA) (10% final concentration of TCA) for 60 min at 4 °C. Cell density was determined by the Sulforhodamine B assay (SRB) [37]. The medium was removed and the plates were washed four times with water. Then 50 μL of SRB (0.1% (w/v) in 1% acetic acid) was added to each well, and the plates were incubated for 30 min at room temperature. The excess dye was removed by washing repeatedly with 1% (v/v) acetic acid (150 μL per well) and the plates were air-dried. The protein-bound dye was dissolved in 10 mM Tris base solution, and the absorbance was determined at 540 nm using a microplate reader. Modification of growth was expressed as a percentage gain or loss relative to the control using the following equation:

$$\textbf{Growth rate} = (\text{absorbance in test well}/\text{absorbance in control well}) \times 100\%$$

2.5. Adhesion assays

Adhesion to SW480 cells of propionibacteria coming from reaction mixtures with lectins was assessed as previously described [35]. Bacterial cells were resuspended in D-MEM/F12 without antibiotics at an initial concentration of approximately 1×10^8 CFU/mL. SW480 monolayers developed in 24-well-plates were washed three times with Dulbecco's PBS buffer to remove antibiotics, and then 1 mL of bacterial suspensions (10^8 CFU/mL) was added to each of the three wells containing 10^7 cells/mL (a ratio of approximately 10 bacteria: 1 eukaryotic cell). The plates were incubated for 1 h at 37 °C in a 5% CO_2 atmosphere and then the monolayers were washed three times with sterile saline solution to remove unbound bacteria. Afterwards, the monolayers were detached and disrupted with 1 mL of a solution containing 0.5% (v/v) Triton X-100 and 025% (v/v) trypsin in PBS. The resultant lysates were transferred to tubes containing 9 mL of PBS, serially diluted and plated on MRS agar incubated at 37 °C in anaerobiosis for 120 h. The results were expressed as the percentage of bacteria adhered (P_1) with respect to the amount of bacteria added (P_0): [(P_1/P_0) x 100].

2.6. Growth in media with lectins as the sole energy sources

A suspension of active propionibacteria (obtained as described in Section 2.2) was incubated at 37 °C for 3 h to deplete cells of intracellular reserves. The suspension was inoculated at a rate of 2% (v/v) in a Chemical Defined Medium (CDM) [38] supplemented with 2, 10, 50 and 100 μg/mL of ConA or AIL with or without 2.5 g/L glucose or galactose, respectively, and incubated at 37 °C without shaking for 80 h. Growth was followed by absorbance at 560 nm.

2.7. Preparation of cecal homogenates and in vitro fermentation assay

Six-week-old Balb/c male mice, each weighing 25–30 g, were used as the source of colonic microbiota for intestinal fermentation assays. The animals obtained from the inbreed colony kept at CERELA were housed in metal cages under controlled environmental conditions of 25 °C and a 12:12 h light–dark cycle with free access to water and a conventional solid balanced diet with the following composition (g/kg): water, 120; proteins, 230; carbohydrates, 538; lipids, 50; vitamins, 22; minerals, 40 (CARGILL, Molinos, Entre Ríos, Argentina). On the day of the experiment, nine mice were sacrificed by cervical dislocation, and their large bowels were carefully removed and introduced into an anaerobic glove box (Anaerobic System model 1024, Forma Scientific, Marietta, USA) with an atmosphere of 85% N_2, 5% CO_2 and 10% H_2. The intestinal contents were pooled, weighed and diluted in pre-reduced sterile saline solution (0.9% w/v NaCl) to obtain a 10% (w/v) suspension [31]. Aliquots of this homogenate were transferred to sterile glass tubes and then supplemented with sterile saline solution (control homogenate), ConA (400 μg/mL), AIL (400 μg/mL), propionibacteria (approx. 1×10^{10} bacteria/mL), propionibacteria plus each lectin, lectins plus glucose (5 g/L) and propionibacteria plus lectins and glucose to obtain ten different fermentation mixtures. After 15 h of incubation at 37 °C in the anaerobic chamber, suspensions were finally centrifuged (1000× *g*; 10 min; 4 °C) and the supernatants were frozen for further analysis. The experimental protocol used in this study was approved by the Ethical Committee of CERELA (CRL-BIOT-EF-2014/2A).

2.8. Organic acids analysis

Samples taken for organic acids analyses were deproteinized with H_2SO_4 0.01 mol/L (15 min, 4 °C), centrifuged for 10 min (10,000× *g*; 4 °C) and filtered onto 0.2 mm pore size membranes (Millipore, Massachusetts, USA). SCFA produced during fermentations were determined with an HPLC system (equipped with Smartlinepump 100, refractive index detector K-2301 and smart line autosampler AS 3800, Knauer, Germany) using a 300 × 7.8 mm Rezex ROA organic acids column (Phenomenex, Torrance, USA) operated at 55 °C with H_2SO_4 0.01 mol/L as the mobile phase at a 0.6 mL/min flow rate. Product concentrations were reported as g/L of cecal suspensions.

2.9. Statistical analysis

The results are expressed as the average of three independent experiments. Significant differences between means were determined by Tukey's test after analysis of variance (one-way ANOVA) with Minitab Statistic Program, release 12 for Windows. A P value of <0.05 was considered statistically significant.

3. Results and discussion

Vegetarian diets, as well as those with limited access to animal protein due to economic or cultural reasons, include many food items such as beans, cereal grains, nuts, seeds and fruits, that may contain besides nutrients some anti-nutritional factors such as protease inhibitors, phenolic compounds (tannins), phytic acid and lectins, among others [3]. Lectins are nonimmune proteins or glycoproteins that bind specifically to carbohydrate moieties expressed on the cell surface affecting their physiology in beneficial or detrimental ways according to the dose, the duration of exposure and other factors such as the glycosilation status of the epithelium [9]. Some lectins are mitogenic and influence human colonic epithelial cell proliferation [8,18–22,39,40]. This property may be relevant in some intestinal inflammatory and neoplastic conditions, in which lectin receptor expression is increased, allowing interaction with dietary lectins which would otherwise pass through the normal colon without binding [18,22,39,40].

In the present study, all lectins tested were able to affect, in a different manner, the growth of SW480 colon cancer cells. Lectins showed dose-behavior and the greatest effects generally resulted from the highest concentrations tested (Fig. 1). Only WGA inhibited

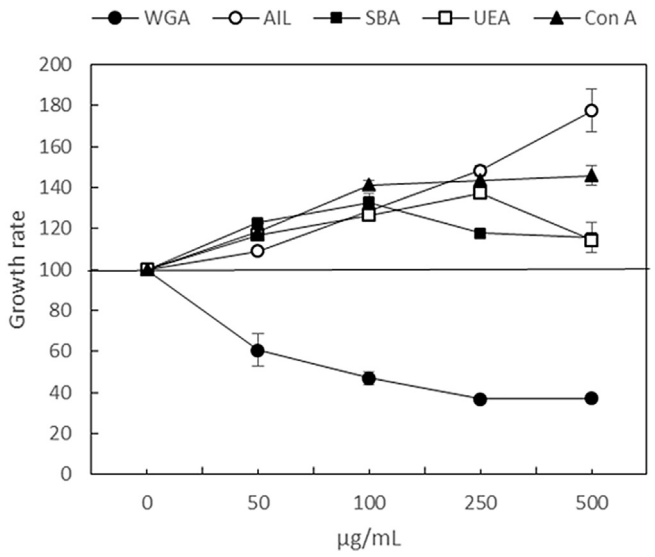

Fig. 1. Effect of plant lectins on cell growth of SW480 human colorectal cancer cell line. The influence of four concentrations (50, 100, 250 and 500 µg/mL) of five lectins: WGA (wheat germ agglutinin); AIL (*Artocarpus integrifolia* agglutinin); SBA (soybean agglutitnin); UEA (*Ulex europaeus* agglutinin); ConA (concanavalin A) on cell growth was determined by the sulforhodamine B assay at 48 h after addition of lectins and compared with control conditions (without lectins). Each experimental condition was replicated six times. The control condition of culture is represented by the horizontal line labelled as 100%.

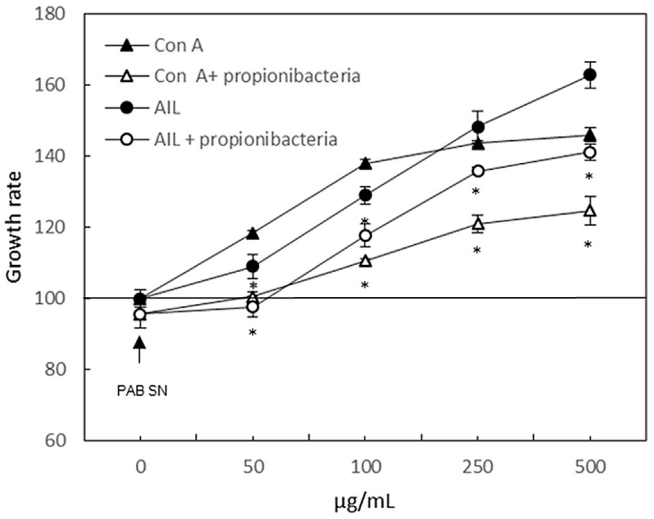

Fig. 2. Growth of SW480 cell line after exposure to supernatants of co-incubation of lectins with propionibacteria. Four concentrations (50, 100, 250 and 500 µg/mL) of two lectins (AIL: *Artocarpus integrifolia lectin* and ConA: concanavalin A) were incubated with *P. acidipropionici* CRL 1198 and then supernatans were added to growth media of colonic cells. Cell growth was determined by the sulforhodamine B assay at 48 h after addition of lectins and compared with control conditions (without lectins). The arrow shows the effect of propionibacteria supernatant alone (PAB-SN). The asterisks indicates differences with their respective control ($P < 0.05$).

growth of colonic cells in all concentrations assayed, whereas the other lectins increased proliferation in relation to control cells grown without them.

Other authors have reported previously, that there is an inhibitory effect of WGA on different cancer cell lines such as HCT-15, LoVo, SW837 and HT-29 at 10–100 µg/mL concentrations [18,20]. Both SBA and UEA increased proliferation, showing the highest effects at 100 and 250 µg/mL, respectively. Since AIL and Con A stimulated growth in all the concentrations tested in a dose-dependent manner, these lectins were selected for further assays. In a previous study, the same lectins showed remarkable cytotoxicity on normal colonic epithelial cells as well as an *in vitro* interaction with the potential probiotic *P. acidipropionici* CRL 1198 [34]. Therefore, propionibacteria were incubated for 60 min at 25 °C with the different lectins concentrations, and the supernatants of reactions were added to the colonic cell line in order to assess their effects on growth. As seen in Fig. 2, the reaction of propionibacteria with both lectins decreased the proliferative effects of supernatants in a dose-related manner, suggesting some specific removal of these compounds by bacterial cells. Control SW480 cells incubated in DMEM/F12 showed no significant difference in growth with cells exposed to supernatants of propionibacteria without lectins (Fig. 2, arrow). It has been reported that different lactic acid bacteria exert antiproliferative effects on adenocarcinoma colon cancer cells [41], but this strain of propionibacteria did not show this kind of effect. Interaction of propionibacteria with 50 µg/mL of ConA or AIL completely abolished the proliferative effects of lectins on colonic cells, whereas the reaction with higher lectin concentrations decreased the stimulation of growth exerted by each lectin alone from 4.43 to 27.31%.

In a previous study, a significant loss of viability of normal colonocytes was observed when they were incubated with 100 µg/mL lectins, but the cellular damage was significantly decreased when lectin solutions were preincubated with bacteria [34]. In a similar manner, Babot et al. (2016) [42] observed that soybean lectin (SBA) was cytotoxic for intestinal epithelial cells (IEC) of chicks, but this effect could be prevented by lectin binding on the envelope of *Bifidobacterium animalis* CRL 1395. In the present study, the reaction of lectins with *P. acidipropionici* CRL 1198 was shown to inhibit growth of the adenocarcinoma cell line. Therefore, it seems evident that microorganisms are able to remove these compounds from the media avoiding their undesirable effects on cells. Bacteria from interaction mixtures were assayed for their ability to adhere to SW480 cells. In agreement with the supernatants effects, adhesion showed a pattern related to lectins concentration, since propionibacteria coming from the reaction with 500 µg/mL of lectins adhered less than those reacting with 50 µg/mL (Fig. 3).

When the hapten sugars (D-mannose for ConA and N-acetyl-D-galacotsamine for AIL) were included in the reaction mixtures containing 100 µg/mL of lectins, adhesion of propionibacteria demonstrated a lower decrease compared to PAB + lectin ($P > 0.05$), suggesting that specific reactions between sugars and lectins leave more bacteria able to adhere to epithelial cells (Fig. 4).

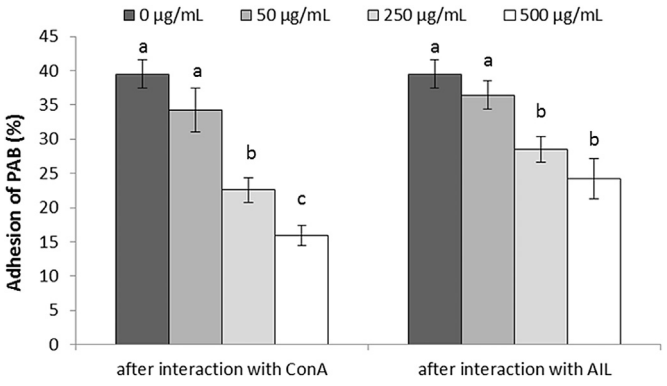

Fig. 3. Percentage of adhesion (bacteria adhered with respect to the amount of bacteria added) of *Propionibacterium acidipropionici* CRL 1198 to SW480 colon cancer cell line. Results presented are the mean ± SD of cells bound for six wells from two independent experiments. Bars sharing the same letter are not significantly different ($P > 0.05$). ConA: concanavalin A, AIL: *Artocarpus integrifolia* lectin.

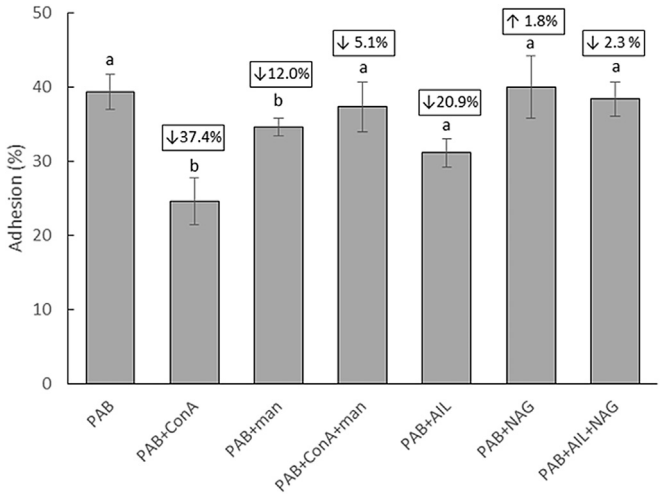

Fig. 4. Adhesion of *P. acidipropionici* CRL1198 (PAB) to SW480 cell line after incubation with 100 μg/mL of concanavalin A (ConA) or *Artocarpus integrifolia* lectin (AIL) with or without simultaneous presence of 50 mmol/L D-mannose (man) and N-acetyl-D-galactosamine (NAG), respectively. Each bar represents mean ± sd of the three data from two assays. The square box above each bar shows the change in adhesion as compared to PAB alone taken as control. Bars sharing the same letter are not significantly different ($P > 0.05$).

This hapten inhibition test confirmed that removal of lectins was due to a surface phenomenon that involves specific recognition and reaction between lectins and carbohydrate structures placed on bacterial cell walls. These carbohydrates seem to be part of adhesins involved in the attachment of bacteria to the intestinal mucosa.

Adhesion is a desired property for probiotic microorganisms because it prolongs their persistence in the gut. However, to be considered as probiotics, microorganisms have to exert a health benefit no matter whether or not they adhered to the intestinal mucosa. Then, it would be beneficial for the host if the propionibacteria loses their ability to adhere by binding undesirable lectins to their entire cell surface and being washed out of the gut with the intestinal contents. However, bacterial adhesion is a complex process that may involve other adhesins not complimentary to lectins. In previous studies we observed that *P. acidipropionici* CRL 1198 adheres to intestinal cells by adhesins of a different nature [43] so that it could be expected that adhesion decreases but does not disappears after the reaction with lectins. In addition, we observed that bacterial adhesins and the sites for lectins attachment on the bacterial surface were related in *P. acidipropionici*, as the property to adhere to IEC and the ability of this strain to remove ConA were both decreased after treatments with periodate and pronase E [34]. As observed with normal colonic cells, *P. acidipropionici* pre-incubated with ConA showed less adhesion to SW480 adenocarcinoma cells. Since adhesion was not abolished, it could be assumed that carbohydrates other than glucose and mannose on the bacterial surface as well as other surface structures are also involved in the bacteria-colonic cell interaction. However, the cell wall composition of dairy propionibacteria, particularly its carbohydrates contents, supports their interaction with ConA and AIL (it is known that *P. acidipropionici* possess glucose, mannose, N-acetylgalactosamine and galactose which would explain the removal of Con A and AIL) [41,44]. Recently, Babot et al. (2016) [42] also observed that the percentage of enterocytes with adhered *B. infantis* CRL1395 significantly decreased from 50% to 17% after incubation of bacteria with SBA, which supports the hypothesis that bacteria would bind lectins in the intestinal lumen and, by losing part of their ability to adhere to IEC, would be eliminated, carrying the lectin attached to its surface along with the normal transit of digesta. It would be interesting to determine if bacteria that are still able to adhere (with or without lectins bound to them) can protect the host from remnant lectins or exert any other beneficial effects. In an *in vivo* study, we observed that the intestinal mucosa of mice fed the strain CRL 1198 at the same time as ConA had lower counts of propionibacteria compared to the group that received only PAB but also had fewer structural and functional alterations of the mucosa than those fed only with lectin [12]. Further studies are needed to clarify the mechanism involved in the observed effect. To determine whether the decrease in lectin-induced SW480 cells proliferation was due only to binding or includes metabolism of lectins by propinibacteria, *P. acidipropionici* CRL 1198 was cultured in a chemical defined medium (CDM) containing ConA or AIL as the sole carbon and energy source (Fig. 5). No growth was observed in the presence of lectins in concentrations ranging from 2 to 100 μg/mL of ConA or AIL, so production of SCFA was null. When glucose or galactose was included in the culture media, propionibacteria were able to develop, but lectins inhibited growth in a dose-dependent manner (which could be related to sugar-lectins binding being less available for microbial growth) (Fig. 5). The results suggest that propionibacteria decrease lectins bioavailabilty only by surface binding of these compounds on the bacterial envelope.

Intestinal microbiota has an important role in host physiology since it is involved in the breakdown and bioconversion of dietary and endogenous components that are not degraded and absorbed by the digestive system. The end-products generated by microbial metabolism fuel enterocytes and exert signaling functions that induce systemic immune and metabolic responses related to body homeostasis. Then, the optimal metabolic activity of gut microbiota seems to be crucial for the maintenance of host health and prevention of disease. Short chain fatty acids (SCFAs), are the main bacterial metabolites produced by the fermentation of dietary fiber, resistant saccharides and residual proteins by specific colonic anaerobic bacteria. In particular, acetate, propionate and butyrate represent 85–95% of total SCFAs present in the colon and are involved in a wide range of physiological functions, such as the transport of electrolytes and water, growth, differentiation and apoptosis of colonocytes, metabolism of lipids in the liver and energy supply for different tissues [45]. Recent studies have also highlighted that their deficiency may affect the pathogenesis of several diseases, such as allergies, asthma, and cancers, as well as autoimmune, metabolic and neurological diseases. It has been reported that some dietary lectins such as PNA, produce changes in intestinal microbiota composition [46], such as overgrowth of enterobacteria in the intestine, which in turn could increase the production of undesirable metabolites associated with their metabolism [14]. In the present study, the addition of ConA to colonic homogenates of mice used as a model of colonic microbiota inhibited fermentation of endogenous substrates since SCFA production decreased in relation to control without lectins (2.39 ± 0.22 vs. 2.95 ± 0.13 g/L, $P < 0.05$) (Fig. 6), whereas AIL showed no significant effect (data not shown). Different studies have reported that plant lectins of different carbohydrate specificities are able to promote growth inhibition or death of bacteria and fungi. This antimicrobial effect has been ascribed to inhibition of nutrient absorption, blockage of sugar metabolism, and cytotoxic effects due to morphological alterations that include the presence of pores in the Gram-positive bacteria membrane and bubbling on the Gram-negative bacteria cell wall [47]. Since microbiota was not quantified in the present study, we can only speculate that fermentation inhibition could be due to either growth inhibition or cytotoxicity exerted by lectins and that a lower number of viable and active microorganisms present in the cecal contents resulted in a

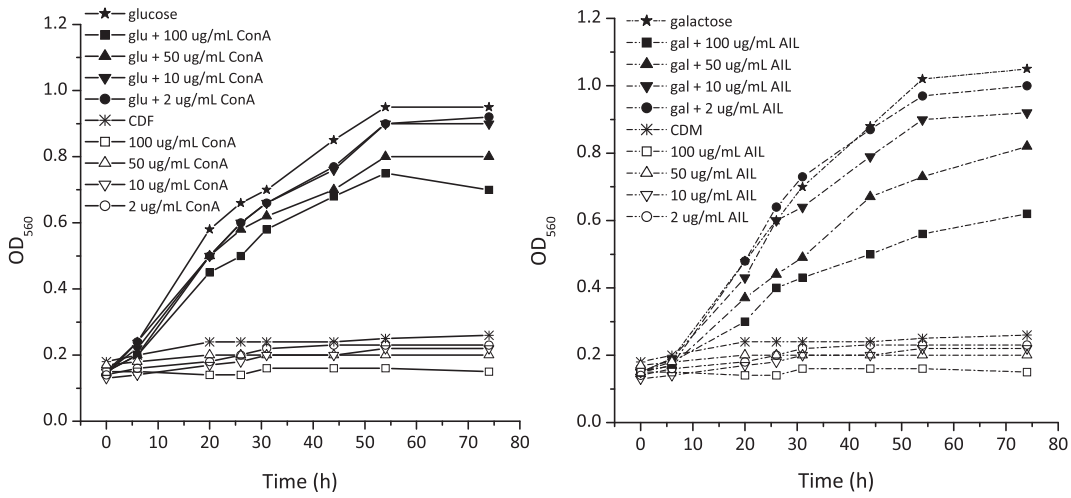

Fig. 5. Growth of *P. acidipropionici* CRL 1198 in chemical defined medium (CDM) with different concentrations of ConA (concanavalin A) (panel A) or AIL (*Artocarpus integrifolia*) (panel B) with or without glucose (glu) or galactose (gal) respectively as additional energy source.

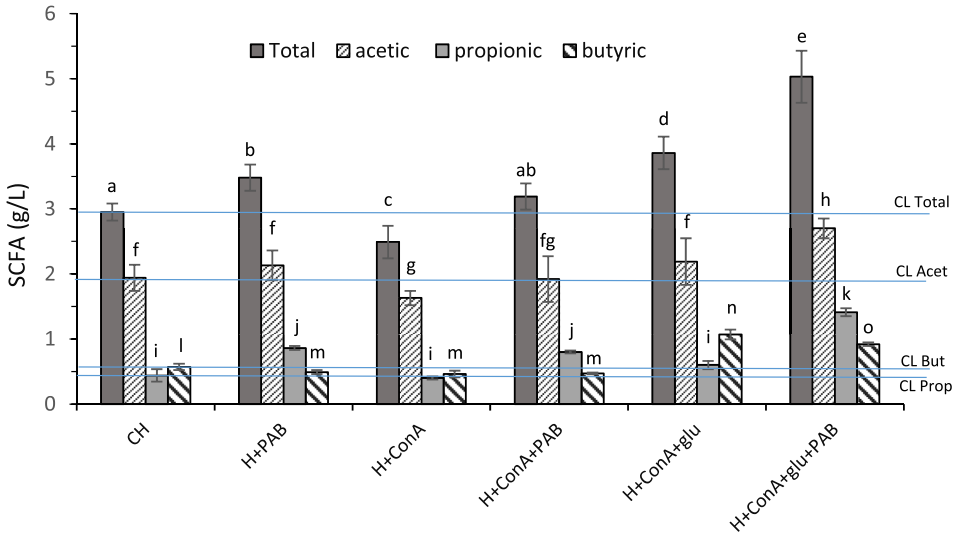

Fig. 6. Production of short chain fatty acids by colonic homogenates in the absence/presence of concanavalin A (ConA), propionibacteria (PAB) and glucose (glu). Samples were incubated 15 h at 37 °C in anaerobic conditions and acetic, propionic and butyric acid and total short chain fatty acids (SCFA) were quantified. Results are expressed as means ± sd. Horizontal lines indicates control level (CL) of acids for easier comparison. Each column was compared with its respective control. The same lowercase letter on columns indicates values not significantly different ($P > 0.05$).

decreased production of SCFA. Homogenate supplemented only with *P. acidipropionici* CRL 1198 contained, after 15 h of incubation, more SCFA than the control (3.48 ± 0.20 vs. 2.95 ± 0.13 g/L, $P < 0.05$), suggesting that propionibacteria were viable and able to efficiently metabolize endogenous substrates. This modification was mainly due to the increase in the propionic acid's ratio at the expense of butyric acid, whereas no changes in the relative amounts of acids were observed in the case of ConA supplementation (Fig. 6, Table 1). Fermentation inhibition exerted by ConA was prevented by the inclusion of propionibacteria in the homogenates (Fig. 6), whereas the addition of glucose as supplementary energy source also modified fermentative activity of colonic microbiota by increasing total SCFA due to butyric acid production at the expense of acetic acid (Fig. 6, Table 1). Finally, homogenates supplemented with propionibacteria and glucose at the same time as ConA contained significantly higher amounts of SCFA than the other groups, with increases in the three volatile fatty acids. Average concentrations of 2.7, 1.41 and 0.92 g/L for acetic, propionic and butyric acids, respectively, were detected in this homogenate but the relative amounts of acids showed differences related to the sample containing glucose but no PAB; that is, while acetic and

Table 1
Relative amounts of acetic, propionic, and butyric acids in mice cecal homogenates supplemented or not with concanavalin A (ConA), propionibacteria (PAB) and glucose (glu).

Experimental condition	Molar ratio A:P:B[a]
CH	65.8:14.9:19.3
H + CRL 1198	61.2:24.7:14.1
H + ConA	65.5:16.1:18.5
H + ConA + CRL 1198	60.2:25.1:14.7
H + ConA + glu	56.7:15.5:27.7
H + ConA + glu + CRL 1198	53.7:28.0:18.3

[a] A, acetate; P, propionate; B, butyrate (Mean value of % of total SCFA).

butyric acids' molar ratios diminished, the relative amount of propionic acid increased (Table 1). Previously, we reported that homogenates inoculated with strains of *P. acidipropionici* produced more propionic acid and less butyric acid from sucrose fermentation than homogenates without the addition of PAB and that the relative amount of acetic acid was also lower, although propionibacteria are acetic acid producers [31].

Due to the broad metabolic capacity of intestinal microbiota and its high plasticity, several attempts have been made to modulate colonic fermentation by dietary manipulation, such as the consumption of probiotic *Lactobacillus* and *Bifidobacterium* strains [48–50]. Among dairy bacteria, propionibacteria seem to be better candidates for increasing SCFA production within the intestine, since they themselves are propionic and acetic acid producers. Previous studies have shown that *P. acidipropionici* CRL 1198 was able to remain viable and functionally active and modify colonic fermentation of lactose, sucrose and fructooligosaccharides by producing a greater amount of SCFA [31,36].

Different attempts have been made to prevent or counteract the toxic or anti-nutritional effects of dietary compounds on the gastrointestinal tract, including suitable diets. Regarding lectins, other authors have postulated that a high dietary intake of complementary sugar-containing carbohydrates would offer protection from their deleterious effects by binding free lectin in the colonic lumen [20,21,51]. In this sense, Ramadass et al. (2010) [51] have reported that the administration of sucrose to rats reduced the toxic effects of legume lectins, whereas others have proposed that a high intake of galactose-containing vegetable fiber would offer protection against binding and proliferative effects of galactose-N-acetylgalactosamine-binding dietary lectins (such as PNA) on colonic neoplastic epithelium [21]. Since the administration of high doses of sugars could produce undesirable changes in intestinal fermentation, other alternatives deserve consideration. In this respect, we propose that a similar role could be played by bacteria with suitable sugar moieties on their surfaces, which would decrease the interaction between dietary lectins and colonic cells by competing for the sites where these molecules bind, by blocking receptors on the mucosa surface or by washing out these compounds from the gut attached to their cell walls. This should prevent the toxicity of lectins on IEC and their anti-nutritional effects for humans and animals. Having this concept in mind, we have selected propionibacteria that could be consumed as a part of human or animal diets to decrease the interaction between lectins and intestinal epithelial cells. In previous *in vitro* studies, we observed that *P. acidipropionici* CRL 1198 was able to bind and remove different dietary lectins from the medium decreasing their cytotoxicity on exfoliated colonocytes [34]. When the strain was consumed daily with ConA by Balb/c mice, it prevented the decrease in food efficiency, the enlargement of organs, the disruption of brush border membranes and the inhibition of disaccharidase activities caused by lectin [12]. These positive effects could be attributed to a barrier effect of propionibacteria adhering to the mucosa [33,35] and/or to the binding of free lectin within the intestinal lumen contributing to a reduction in the amount of free lectin able to interact with the mucosa [34]. It could be expected that almost 4 mg of lectin should be adsorbed by 10^9–10^{10} propionibacteria, an amount that is possible to find in the cecal content of mice fed with diets containing some strains of these bacteria [31].

Other authors have reported the ability of dairy propionibacteria to bind and remove toxic compounds such as aflatoxins *in vivo* in the intestinal lumen of chicken [52]. In the same manner, the early administration of *B. infantis* CRL1395, a strain expressing N-acetyl-D-galactosamine on its surface, to chicks has been proposed to reduce the toxicity of SBA and provide the birds with putative probiotic bacteria in the first days after hatching [42]. In contrast, probiotic *Lactobacillus plantarum* 299v fed to rats did not counteract the unfavorable phytohaemagglutinin-induced changes in their intestinal microbiota [46].

In conclusion *P. acidipropionici* CRL 1198 decreased the proliferative effects of lectins on adenocarcinoma cells and the inhibition of colonic microbiota fermentative activity and may be considered as a tool to avoid undesirable lectin-epithelia-microbiota interactions. Further investigations on their potential as probiotic protecting agents against dietary toxic lectins both in humans and animals are ongoing.

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