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Chemotactic responses of the rumen bacterial community towards the daidzein flavonoid

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ABSTRACT

Chemotaxis is a mechanism which involves bacterial mobilization to find nutrients or escape from harmful environments. Although ruminal fermentation processes and its by-products are well-known, rumen bacterial chemotaxis has received no attention. Daidzein is one of the common metabolites in plants and has chemotactic effects on soil bacteria that colonize the plants. There are several tests to assess bacterial chemotaxis, but none focused on anaerobic microorganisms as rumen bacteria. We standardized a chemotaxis assay for rumen bacteria by modifying a well-known aerobic capillary method by combining it with technology commonly used for measuring *in vitro* gas production. Parallel assays were included for studying daidzein isoflavone as a possible attractant and the effect of different chemoattractants (sterile rumen fluid, cellulose and daidzein) on ruminal bacterial consortium was tested. Daidzein showed 3 phylotypes (phylotypes 1, 3 and 4); phylotype 3 was also present in the rumen fluid and cellulose, phylotype 1 (present only in daidzein) was identified as a microorganism closely related to *Ruminococcus albus* 7. A better understanding of the mechanisms underlying rumen microbial fermentation can lead to a proper manipulation in order to create probiotic cultures for cattle, which could act beneficially on the intestinal flora of the individual.

1. Introduction

The rumen is a highly dynamic environment, and none of the changes are permanent within the influence due to microbial species (bacteria, archaea, fungi and protozoa), which are found in the rumen, since each species has an affinity for a substrate and/or fermentation by-products (Janssen and Kirs, 2008). The potentially bioactive compounds found in plants which constitute ruminant diet

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have become an area of interest in animal nutrition (Hammes and Hertel, 2002). Recent studies have shown that extracts of plants containing secondary metabolites (i.e. saponins, tannins and essential oils) can modify rumen microbial population. The biochemical content of plants is part of the diet of ruminants and these bioactive components (polyphenols, phytoestrogens, glycoalkaloids) are interesting in the rumen nutrition (Varel et al., 1991; Hammes and Hertel, 2002). Numerous studies suggest the possibility of using them as natural food additives to improve the efficiency of ruminal fermentation, increase protein production, and decrease methane production (Wang et al., 2000; Muetzel et al., 2003; Patra et al., 2006).

A rapid binding of ruminal bacteria to the recently ingested forage is essential for efficient fermentation and utilization of nutrients. Pell and Schofield (1993) called the *Phase 1*: "the transport of bacteria to the substrate" as a chemical dialog conducted between plant and rhizobacteria. Plant roots secrete a wide range of compounds, among those sugars and amino acids are attractants (chemotaxis) of the microbes, and flavonoids act as signaling molecules to initiate interactions (Drogue et al., 2012; Galicia Jiménez et al., 2011b).

Chemotaxis is a mechanism by which the bacteria respond quickly and efficiently to an attractant concentration gradient, either towards (positive chemotaxis) or away (negative chemotaxis) from such a compound (Murialdo et al., 2009). Bacterial chemotaxis represents one of the simplest and best studied examples of unicellular behavior. The ability of bacteria to rapidly sense and adapt to environmental changes plays a major role in structuring microbial communities, in affecting microbial activities, as well as in influencing various microbial interactions with the surroundings. There are few studies on ruminal chemotaxis, with most focused on ruminal protozoa such as the results reported by Diaz et al. (2014) on chemotaxis responses' comparison between different protozoa when using glucose, xylose and peptides (soy, bacterial lysis, lysis of protozoa) as attractants. Isotrichid protozoa showed a remarkable chemotactic response towards increasing glucose concentration when the cow was fasted (Diaz et al., 2014). Entodiniomorphids exhibited chemotactic response towards peptides dose regardless of peptide source (Diaz et al., 2014); this confirms its leading role in ruminal proteolysis. Hook et al. (2012) emphasize that the attached protozoal population is a significant component of the total rumen protozoal community. To maintain their numbers in the reticulo-rumen, protozoa can be selectively retained through association with feed particles and the rumen wall. Orpin (1985) suggests that chemotaxis plays an important role in the association of rumen ciliate populations with food particles.

Daidzein is one of the most common isoflavonoid in plants, and it has chemoattractant effects on soil bacteria, which colonize plants (Gough et al., 1997; Peck et al., 2006). Previous reports suggest that there is an interaction between rumen microorganisms and daidzein (Mao et al., 2007). In addition, this isoflavones may have the potential to be used as a prebiotic substance in animal feed (Yao et al., 2004a, 2004b).

Many aspects remain to be clarified to understand how bacteria sense and respond in the rumen environment. Therefore the aim of this work was to elucidate the chemotactic effect of daidzein over the rumen bacteria.

2. Material and methods

2.1. Chemotaxis assay

2.1.1. Culture medium (artificial saliva)

The medium was prepared according to Menke and Steingass (1988). It consisted of various components: trace minerals solution, buffer solution, a solution of macrominerals, cysteine as reducing solution and resazurin as an indicator of anaerobiosis, prepared under CO_2 (100%).

2.1.2. Obtaining rumen fluid inoculums

The rumen fluid content (solid and liquid) was collected from 3 fistulated cows with 12 hours of fasting, placed in sealed plastic bags (to maintain anaerobiosis) and transported at 39 °C to the Nutrition Laboratory (100 m away), Faculty of Veterinary Medicine, Autonomous University of Yucatán, México. Ruminal fluid of 3 cows was leaked (together) using sterile cheese cloth and collected in a beaker under constant flow of CO_2.The remaining solids were blended under CO_2 (100%) for 20 seconds with artificial saliva in a volume equal to that extracted as rumen liquor (ratio 1:1) (Menke and Steingass 1988). Rumen solids were filtered again and liquor added to the initial rumen liquid obtained. Then, rumen liquor was placed in 45 ml Falcon tubes and centrifuged at 10,000 rpm (16,770*g*)min at 4 °C. The pellet containing the bacteria was recovered and resuspended in 80 ml of artificial saliva medium and incubated at 39 °C overnight. All procedures were carried out under constant flow of 100% CO_2.

2.1.3. Chemotaxis assay

The capillary method of Adler (1973) was adapted to material for measuring *in vitro* gas production (Theodorou et al., 1994). Then, 50 mg/L daidzein (standard Sigma-Aldrich, Mexico, DF, Mexico) (attractant tested), 50 mg/L of cellulose (positive control), artificial saliva (negative control), and rumen fluid (positive control) were prepared. These solutions (daidzein, cellulose, rumen fluid and artificial saliva) were sterilized by filtration (0.22 μm pore size). Then, capillaries (75 mm length, 1.1–1.2 mm and 1.5–1.6 mm inside and outside Ø respectively, Marienfeld, Germany) were filled with 60 μl of the solutions and one end placed inside a serum bottle (100 ml nominal capacity) and one end was kept outside. Capillaries were sealed with clay and inserted through the septum until the open end was in contact with the culture media (80 ml) containing rumen bacteria (Fig. 1). The septa were sealed with parafilm "M" and incubated at 39 °C for 1 h. Subsequently, bacteria that had been attracted by the flavonoid and entered the capillary tubes were transferred into individual sterile eppendorf tubes and transported in a cooler at 4 °C to the Laboratory of Biotechnology, Faculty of Chemical Engineering, University of Yucatan. Samples were centrifuged for 30 min at 13,000 rpm (28,341*g*), and then the cell pellet was resuspended in 30 μl sterile distilled water. 15 assays were done for each attractant