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Novel probiotic evidence of lactobacilli on immunomodulation and regulation of satiety hormones release in intestinal cells

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ABSTRACT

Lactobacilli strains with probiotic traits were tested for their ability to survive to the digestion process modelled during this study. These strains managed to sustain the harsh conditions of the gastric and duodenal phases and showed good adhesion capacities to human Caco-2 cell line. These probiotic microorganisms have survived during these steps, exposed to low pH, high concentration of bile salts and enzymes occurring in the digestion and virtually reached the duodenal compartment in sufficient amount with limited population loss. These lactobacilli strains appeared to be non-cytotoxic after contact with Caco-2 cells for 24 h. Importantly, some of these strains showed immunomodulatory effect, lowering the pro-inflammatory cytokine IL-8 and promoting secretion of the anti-inflammatory IL-10. Besides, *Lactobacillus gasseri* CMUL34 and *Lactobacillus acidophilus* CMUL67 strains were able to modulate secretion and expression of two intestinal hormones: the Glucagon-Like Peptide 1 (GLP-1) and the cholecystokinin (CCK) in STC-1 cells.

1. Introduction

Gut microbiota is a major constituent of the morpho-functional system including intestinal epithelium and mucosal immune system (Scarpellini et al., 2010). This complex system is continually on a dynamic balance and is responsible of the host local intestinal integrity (Scarpellini et al., 2010). Probiotics are live microorganisms that when consumed in adequate amounts, produce beneficial effects on both digestive and systemic levels. Probiotics are overall safe, acid and bile tolerant, able to adhere and colonize the intestinal tract (Sanders et al., 2010). However, the term "probiotic" should be dedicated to live microbes for which a health benefit has been shown in controlled studies (Gaurner et al., 2008). The most commonly used microorganisms as probiotics are lactic acid bacteria (LAB), including *Lactobacillus*, *Enterococcus*, *Streptococcus* and *Bifidobacterium* species. Nevertheless, further microbial species including *Escherichia coli* Nissle, *Bacillus cereus* and *Saccharomyces cerevisiae* are also recognized as probiotics (Fijan, 2014). Indeed, lactobacilli colonize the gut and vagina of mammalians. They are widely described as beneficial microorganisms because of their aptitudes to relieve irritable bowel syndrome, reduce diarrhoea, promote immunity and restore the balance of gut microflora (Akoglu, Loytvedb, Nuidingb, Zeuzemc, & Faustb, 2015; Aragon, Graham, Borum, & Doman, 2010; Galdeano & Perdigón, 2006; Mitsuoka, 1996; Orel & Kamhi-Trop, 2014; Van Niel, Feudtner, Garrison, & Christakis, 2002). Moreover, lactobacilli play an important role

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in the protection of the host against harmful microorganisms and also strengthen the immune system (Soccol et al., 2010). Some species were described to improve food digestibility and reduce metabolic disorders (Kumar et al., 2012). LAB and particularly lactobacilli were used, since centuries, for food preservation and food fermentation as beneficial agents. LAB are able to modify physicochemical properties of food by fermentation, enhancing their taste and their digestibility and to prevent contamination of potential pathogens by lowering the pH (Gaurner et al., 2008). The genus *Lactobacillus* is commonly found in the upper gastrointestinal tract of mammals. To express their beneficial effects in the host, probiotic strains must be able to survive the passage through the digestive tract. Tolerance to high concentration of bile salts is an important parameter to microbial survival in the intestinal tract. So far, research has mainly focused on strains sensitivity toward low pH, proteolytic enzymes and bile salts (Sanders et al., 2010). In several reports, human enterocyte-like Caco-2 cells have been used for *in vitro* studies to elucidate the mechanisms of cellular adhesion of nonpathogenic lactobacilli (Chauvière, Coconnier, Kernéis, Fourniat, & Servin, 1992; Ferreira, Grzeskowiak, Collado, & Salminen, 2011). The ability of lactobacilli to adhere to mucosal surfaces of the intestine, and the subsequent long or short-term colonization have been the most commonly encountered criteria for the selection of probiotic strains.

Another recent probiotic lactobacilli interest is their action on gastrointestinal hormones involved in food intake regulation. Among these hormones, two are the focus of different investigations: the glucagon like peptide-1 (GLP-1) and the cholecystokinin (CCK) (de Graaf, Blom, Smeets, Stafleu, & Hendriks, 2004). These hormones were described to be involved in the control of appetite as satiation signals secreted by the intestine in response to meal stimulation (Mars, Stafleu, & de Graaf, 2012; Moran & Dailey, 2011; Walsh, 1994). GLP-1 is produced by enteroendocrine L cells, stimulates glucose-induced insulin secretion and inhibits glucagon secretion. This hormone has two active forms, GLP-1 (7–36) amide and GLP-1 (7–37), product of a posttranslational processing of a proglucagon precursor in the mammalian intestine (Oh, Lee, Ko, Choi, & Kim, 2003). CCK is produced in the gut by enteroendocrine I cells and is a member of a peptide hormone family, characterized by the same carboxyl-terminal pentapeptide sequence (-Gly-Trp-Met-Asp-Phe-NH_2). This hormone is widely distributed throughout the central nervous system and the digestive tract (Oikonomou, Buchfelder, & Adams, 2008). The STC-1 plurihormonal cell line is derived from an endocrine tumour developed in the small intestine of a double transgenic mouse (Rindi et al., 1990). The STC-1, murine endocrine cells, are considered a suitable model for the *in vitro* study of gastrointestinal hormones release (Cordier-Bussat et al., 1997, 1998; Sufian, Hira, Asano, & Hara, 2007). These endocrine cells are able to secrete both GLP-1 and CCK hormones (Cordier-Bussat et al., 1997, 1998; Cudennec, Fouchereau-Peron, Ferry, Duclos, & Ravallec, 2012; Geraedts, Troost, Fischer, Edens, & Saris, 2011).

This study provides further probiotics traits to recently proven beneficial lactobacilli isolated from vaginal origin of Lebanese women (Al Kassaa, Hamze, Hober, Chihib, & Drider, 2014). The resistance of these strains to the harsh conditions of the digestion process was evaluated throughout the buccal, gastric and duodenal phases simulated *in vitro*. Then the adhesion capacities of these lactobacilli strains were studied and their cytotoxicity toward Caco-2 cells was determined. Immunomodulatory effect of these strains, particularly on IL-6, IL-8 and IL-10 secretion, was also considered. Finally we look for the impact of these lactobacilli strains on the secretion and expression of CCK and GLP-1.

2. Materials and methods

2.1. Bacterial strains

Lactobacillus strains used in this work were recently isolated by Al Kassaa et al. (2014). These strains were *Lactobacillus gasseri* CMUL34, *L. fermentum* CMUL54, *L. gasseri* CMUL57, *L. acidophilus* CMUL67, *L. gasseri* CMUL80, *L. gasseri* CMUL99 and *L. plantarum* CMUL140. Before each experiment, the strains were grown for 18–24 h at 37 °C in De Man–Rogosa–Sharpe (MRS) medium (De Man, Rogosa, & Sharpe, 1960).

2.2. Lactobacilli tolerance to the conditions of the modelled digestion process

To assess the survivability of the *Lactobacillus* strains, a static *in vitro* digestion model, adapted from Versantvoort, Oomen, Van de Kamp, Rompelberg, and Sips (2005) research was used in this study. This model reproduces temperature, pH, bile salts concentration and enzymes involved in the digestion process in gastrointestinal (GI) environment. Indeed, mouth, stomach and small intestine steps of digestion were simulated using substitution fluids mimicking the physiological conditions of each step (Table 1). After 18 to 24 h of culture in MRS medium, *Lactobacillus* cells were collected by centrifugation at 8000*g*, 4 °C, for 10 min. The pellets were washed twice in adequate volume of Hepes buffer without glucose (NaCl 140 mM, Hepes 20 mM, KCl 4.5 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1.2 mM, adjusted to pH 7.4 with 3 M NaOH). Mouth step reproduced buccal conditions with adjusted pH 6.8 to 7 under agitation at 160 rpm, 37 °C for 5 min in a final volume of 4 mL. Upon this step, the gastric step was simulated by addition of gastric fluid containing 1.56 $mg.mL^{-1}$ of porcine pepsin and adjusted to pH 3–3.5. This step of two hours was conducted in the same conditions of temperature and agitation as the previous step (37 °C at 160 rpm). 1 mL of 1 M $NaHCO_3$ solution was added to the batch to increase the pH solution up to 7, stopping pepsin digestion and mimicking the passage from the stomach to the duodenum compartment. Duodenum phase included addition of pancreatic enzyme at 0.28 $mg.mL^{-1}$ and bile fluid containing 60 $g.L^{-1}$ of bile salts. Then the intestinal digestion process was carried out over 2 hours at pH values ranging from 7.0 to 7.5. After each step of the simulated digestion process, samples of each lactobacilli strains exposed were collected and diluted in saline buffer. Then 100 µL of the adequate lactobacilli dilutions were plated on MRS agar plates and incubated at 37 °C for 18 to 24 h to allow sufficient growth. The number of colony forming unit (CFU) by millilitre of each lactobacilli strain was calculated.