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Small surface-associated factors mediate adhesion of a food-isolated strain of *Lactobacillus fermentum* to Caco-2 cells

Loredana Baccigalupi [a], Anna Di Donato [a], Marianna Parlato [a], Diomira Luongo [b], Virginia Carbone [b], Mauro Rossi [b], Ezio Ricca [a], Maurilio De Felice [a,*]

[a] *Dipartimento di Biologia Strutturale e Funzionale, Università Federico II, Napoli, Italy*
[b] *Istituto di Scienze dell'Alimentazione, Consiglio Nazionale delle Ricerche, Avellino, Italy*

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Abstract

In a search for bacteria having putative probiotic activity, we screened a collection of food-isolated microorganisms for the ability to survive at low pH in the presence of bile salts and for the production of antimicrobial compounds active against a number of animal pathogens. Among these, we found a strain that we classified as a member of *Lactobacillus fermentum* sp., and we further investigated its features. This organism was able to adhere to human enterocyte-like (Caco-2) cells with high efficiency as compared to that of a well known indicator strain. Chromatographic analysis indicated that at least two small (less than 3 kDa) factors were involved in mediating the in vitro interaction of *L. fermentum* with Caco-2 cells. Adhesion activity could be abolished by mild treatment of the bacterial cells in buffer and rescued by incubating them with either the same buffer after its use in the treatment or with chromatographic fractions containing each of the two factors, which indicated that these factors were loosely associated with the cell wall and that each of them was sufficient to warrant the adhesiveness of *L. fermentum* to Caco-2 cells. These data are suggestive of a novel mechanism of bacterial adhesion to epithelial cells.

Keywords: Probiotics; Commensal bacteria; Bacteria–Caco-2 cell interactions; Adhesion

1. Introduction

The human gastrointestinal tract (GIT) is normally colonized by a large variety of non-pathogenic bacteria that constitute the so-called endogenous microbiota, and includes at least 400 microbial species, mostly belonging to such genera as *Lactobacillus*, *Streptococcus*, *Clostridium*, *Bacteroides*, *Bifidobacterium* and *Escherichia* [6,20]. In this symbiotic interaction, bacteria provide the host with essential products, such as vitamins, amino acids and organic acids, and form a first line of defense against invasion by pathogenic organisms. These physiological contributions are reciprocated by stable conditions of temperature, pH, osmolarity and food supply for the microorganisms [6,12]. A normal balanced microbiota is therefore important in preventing intestinal disorders as well as microbial infections. For these reasons, there has been much interest in developing probiotic products containing live bacteria able to survive inside the GIT and display positive effects on human health [15]. Among the beneficial effects ascribed to such preparations are the: (i) a reduction in pathogenic infections through competition for adhesion sites with pathogens, and production of compounds with antimicrobial activity; (ii) modulation of the host immune response; (iii) improvement of lactose tolerance; and (iv) reduction of serum cholesterol levels in vivo [17,18].

Lactobacilli and bifidobacteria are the most frequently used bacteria in probiotic products for human consumption and can be found in infant foods, cultured milk, and various pharmaceutical preparations. It is often asserted that the ideal probiotic organism should be isolated from the GIT of

* Corresponding author.
E-mail address: defelice@unina.it (M. De Felice).

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the species to which it will be administered. A more pragmatic approach recognizes that probiotic activity may result from action at sites other than the GIT (such as the mouth or vaginal tract) and therefore bacteria of non-gastrointestinal origin are frequently found in commercial probiotic products.

In order to exert the expected health benefits to the host, an ideal probiotic bacterium should survive transit through the stomach, adhere to epithelial cells and/or to the extracellular matrix in the intestine and, in some cases, colonize and persist in the gut [13–15]. Bacterial adhesion to human epithelial cells is commonly evaluated by in vitro adhesion assays using Caco-2 cells as an intestinal cell model [2,3,9]. Fully differentiated Caco-2 cells have characteristics of mature enterocytes with functional brush border microvilli and apical hydrolases. Upon differentiation, this cell line forms two clearly distinguishable domains, an apical and a basolateral domain separated by tight junctions. Such domains have completely different ultrastructure and functions from the apical domain containing peptidases and disaccharidases and the basolateral domain containing receptors involved in the control of intestinal hydroelectrolytic secretion [10]. The Caco-2 cell line is therefore a convenient model of enterocytes and has been extensively used to study bacterial adhesion and invasion [2,3].

In the present work, we screened a collection of food-derived lactobacilli, searching for organisms with potential probiotic properties. The most promising strain was identified as *L. fermentum*, a species that normally inhabits the human gastrointestinal and urogenital tracts [7]. The adhesion properties of this strain to cultured human epithelial cells were studied and two adhesion factors identified.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Food-isolated *Lactobacillus* strains are listed in Table 1. *Lactobacillus rhamnosus* LGG is a collection strain (ATCC53103) derived from human feces. Indicator bacteria for antimicrobial assays are listed in Table 2. Lactobacilli were grown in MRS broth (Difco), *Listeria monocytogenes* was grown in brain–heart infusion (BHI) broth (Biolife) while all other bacteria were grown in TY (8 g/l NaCl, 10 g/l tryptone, 5 g/l yeast extract) broth. Lactobacilli were grown at 37 °C in microaerophilic conditions while all other strains were grown aerobically at the same temperature. Biochemical tests for lactobacilli classification were performed by the use of API galleries (Biomérieux).

2.2. Bile salt and pH tolerance

Lactobacillus strains (5×10^7 CFU/ml) were incubated at 37 °C in 5 ml of MRS, acidified MRS (pH 2.5) or MRS containing 0.3% Oxgall (Sigma) to evaluate bacterial tolerance to pH values and to bile salt content similar to those found in the stomach and in the upper parts of the intestine, respectively [13]. Growth was monitored by following the optical density at 620 nm (OD_{620}) for 24 h, while the survival rate was measured by plating 100 µl of each culture on an MRS agar plate at the beginning of the incubation time and after 4 h.

Table 1
Growth and survival properties of fifteen food-isolated lactobacilli at low pH or in the presence of bile salts

Name	Species[a]	pH 2.5[b] (growth/survival)	0.3% Oxgall[c] (growth/survival)
LGG	*L. rhamnosus*	−/+	−/+
VL3A	*L. rhamnosus*	−/+	−/+
MRL20	*L. rhamnosus*	−/+++	−/+
IL39	*L. rhamnosus*	−/+	−/+
L38	*L. rhamnosus*	−/+	−/+
I40	*L. rhamnosus*	−/+	−/+
IL6	*L. rhamnosus*	−/+	−/+
BIO-DRL36	*L. fermentum*	−/++	−/+
DRL38	*L. fermentum*	−/+	−/+
VL36	*L. fermentum*	−/+	−/+
MRL28	*L. fermentum*	−/+	−/+
DRL32	*L. fermentum*	−/+	−/+
DRL33	*L. fermentum*	−/++	−/+
BIO-DRL33	*L. fermentum*	−/+	−/+
BIO-DRL40	*L. brevis*	−/+	−/+
L41	*L. brevis*	−/+	−/+

[a] Assigned based on the results of API tests.
[b] As determined in Section 2:, +, up to 10%; ++, from 10 to 50%; +++, more than 50% of survival.
[c] As determined in Section 2: +, up to 15% of survival.

2.3. Antimicrobial activity assay

Antimicrobial activity was determined with the method described by Schillinger and Lüeke [21] with the following modifications: 10 µl of each culture (listed in Table 2) in stationary growth phase were spotted on the surface of an MRS agar plate and the spots air-dried. A 100 µl of an exponential culture of the indicator bacterial strain were mixed with 5 ml of soft agar (0.7%) and poured over the plate. The plates were incubated aerobically overnight at 37 °C and the inhibition halos were measured and expressed in mm.

2.4. Caco-2 cell culture conditions and adhesion assays

The human colonic cell line Caco-2 was obtained from the American Type Culture Collection (Rockville, MD, USA) and routinely grown in 100-mm plastic Petri dishes (Falcon; Becton–Dickinson Biosciences San Jose, CA, USA) in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) heat-inactivated (30 min at 56 °C) fetal calf serum (FCS), 100 U/ml penicillin, 100 mg/ml streptomycin, 0.1 mM non-essential amino acids, 2 mM L-glutamine (complete medium) and incubated at 37 °C in a water-jacketed incubator in an atmosphere of