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SHORT COMMUNICATION

Evaluation of some in vitro probiotic properties of *Lactobacillus fermentum* Strains

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Abstract This study aimed to check the in vitro probiotic properties of eleven *Lactobacillus fermentum* strains previously isolated from fermented dairy products and infant faeces. These cultures were tested for their tolerance to different pH such as 2.0, 2.5, 3.0, 3.5 and 6.5, bile salt hydrolysis and cell surface hydrophobicity. All the strains were persistent at pH 3.5 for 3 h whereas only faecal origin isolates such as *L. fermentum* BIF-19, BIF-20, BIF-18 and MTCC 8711 had shown considerable growth at pH 2.5. The strains NCDC-400, MTCC 8711, BIF-18, BIF-19 and BIF-20 showed slight to intense precipitation zone of bile salt hydrolase activity by agar plate assay. The strain *L. fermentum* BIF-19 exhibited best preliminary probiotic properties was selected for the adhesion to Caco-2 cell lines, which shows similar adhesion to that observed for standard probiotic *Lactobacillus rhamnosus* GG.

Keywords Probiotic · Acid tolerance · BSH · Cell adhesion · Caco-2 · *Lactobacillus fermentum*

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Introduction

The human intestinal microflora harbors the magnanimous microbiome, that comprises beneficial bacteria of which lactobacilli and bifidobacteria are the two key members influencing overall health and well-being of the host. These microorganisms are called probiotics, which means "for life". Lactobacilli have been prominently noticed for their capability to bestow various health benefits to the host and the species such as *Lactobacillus casei, L. rhamnosus, L. acidophilus, L. reuteri,* and *L. fermentum* are used as probiotic (Cerbo et al. 2016). Among the different species, *L. fermentum* is used in a number of ethnic as well as commercial probiotic preparations. Previous studies have shown health-enhancing properties of *L. fermentum* strains such as ME-3 for its antimicrobial and antioxidative properties, RC-14 for altering vaginal flora, VIR-003PCC for enhancing gastrointestinal and respiratory tract illness and ACA-DC 179 for protecting against *Salmonella* infection (Zoumpopoulou et al. 2008; West et al. 2011; Kullisaar et al. 2016).

Recently, the published study indicated that the intake of probiotics improves gut homeostasis and disease condition such as irritable bowel syndrome (IBS). The authors have specifically mentioned and suggested that ingestion of probiotic provide support for beneficial health effects (Veiga et al. 2014). In order for successful probiotics, the putative strain should show good survivability during the intestinal passage and should be resistant to gastrointestinal tract (GIT) conditions, including acidic pH and bile acids (Cerbo et al. 2016). After the ingestion of a probiotic product, bacteria enter the stomach, where hydrochloric acid makes the pH extremely low. Besides, bile salt hydrolase (BSH) activity is one of the potential genetic markers for the selection of probiotic lactobacilli. The BSH

catalyses the hydrolysis of conjugated bile acids to the amino acid residue (deconjugation) which plays a significant role in maintaining the equilibrium of the gut microflora (Taranto et al. 1999; Zhang et al. 2015). Further, the adhesion effects of lactobacilli to the human intestinal epithelial cells is an important characteristic of probiotics, as it colonizes and proliferates in the human intestinal tract and prevent pathogens from occupying the living space by colonization resistance (Daliria and Lee 2015).

By proteomic study, we have previously shown the presence of different proteins in the two strains of *L. fermentum* (NCDC 400 and RS2) which was attributed to their inherent stress tolerance ability. Corresponding to the proteomics data, 131 and 159 novel proteins were identified in *Lactobacillus fermentum* strain NCDC 400 and RS2 respectively, which were involved in bile salt and acid tolerance processes (Parijat et al. 2017; Kaur et al. 2017). These strains specific studies attract the researchers across the world to isolate and study the putative probiotic strains from the different geographical niche. The ultimate goal is to identify the novel endogenous probiotic strains which contain extreme high tolerance to low pH, bile salt hydrolysis and adhesion to hydrocarbons/epithelial cells, so on and so forth. Therefore, the objective of current study was to evaluate the *L. fermentum* strains for basic in vitro probiotic properties, select and recommend best strain for further in vivo experiments.

Materials and methods

Bacterial strains and growth conditions

The *L. fermentum* strains NCDC-400, C-6, C-9, NCDC-156, NCDC-605, NCDC-606 and MTCC-8711 from the dairy origin, KT-85, BIF-18, BIF-19 and BIF-20 from the human faecal origin and proven probiotic culture; *L. rhamnosus* GG (LGG) was obtained from NCDC lab and used as control probiotic. All the cultures were grown in MRS broth (Hi-media Laboratories Pvt. Ltd, Mumbai, India) overnight at 37 °C. Prior to assay, strains were serially transferred three times by inoculating 2% v/v of inoculums in fresh MRS broth with incubation at 37 °C for 18 h (Parijat et al. 2016).

Acid tolerance

Resistance to acidic conditions was tested according to the method of Clark (1997). The active grown cells were harvested by centrifugation, washed with sterile normal saline (0.85% w/v) and resuspended in equal volume of MRS broth with pH adjusted (using 0.1 N HCl solution) to 2.0, 2.5, 3.0 and 3.5 while pH 6.5 was kept as control. One ml of culture was taken from each tube after an interval of 0, 1, 2 and 3 h. The suitable dilutions were prepared and plated on MRS agar and incubated at 37 °C for 48 h. The experiment was repeated three times and the results obtained at different pH and after each time intervals are given as mean ± SD.

Bile salt hydrolase activity

The ability of lactobacilli to deconjugate bile salt was determined according to the bile salt hydrolase (BSH) assay (Taranto et al. 1999). Bile salt plates were prepared by adding 0.5% (w/v) of sodium salts of taurocholic acid (TC), taurodeoxycholate (TDC) and tauroglycocholate (TGC) and 0.37 g/L (w/v) of $CaCl_2$ to MRS agar. The strains were streaked on the agar media and the plates were anaerobically incubated (GasPak^{TM}100 System, BBL Systems, Maryland, USA) at 37 °C for 72 h. All the chemicals used in this study were purchased from Sigma-Aldrich, St. Louis, USA until unless specifically specified.

Cell surface hydrophobicity

Cell surface hydrophobicity was determined by measuring *L. fermentum* strains adhesion to three hydrocarbons (n-Hexadecane, n-Octane and Xylene) according to Rosenberg et al. (1980) protocol. Briefly, the overnight grown cells were harvested (12,000 g for 5 min at 5 °C), washed twice and re-suspended in phosphate urea magnesium buffer (pH 6.5), so as to obtain an absorbance value of 0.7–0.8 at 600 nm. The bacterial suspensions (3 ml) along with 1 ml each hydrocarbons, were incubated at 37 °C for 10 min followed by 2 min of vortexing. The suspensions were kept undisturbed at 37 °C for 1 h to allow phase separation and the hydrocarbon layer was allowed to rise completely. The aqueous phase was removed and the absorbance at 600 nm was measured. The decrease in the absorbance was calculated and the values shown in triplicate and expressed as mean ± SD.

Adhesion of selected *Lactobacillus fermentum* strain to Caco-2 cell line

Aforementioned *L. fermentum* strains were investigated for quantitative binding on Caco-2 cell line, procured from National Centre for Cell Science, Pune, India and cultured in DMEM supplemented with 10% FBS, 2 mmol/l L-Glu, 100 U/ml Penicillin, 30 µg/ml Streptomycin and were incubated at 37 °C containing 5% CO_2. Adhesion assay

was carried as per the methods described by Jacobsen et al. (1999) protocol. Briefly, the Caco-2 cells were grown in six-well tissue culture plates. The initial number of Caco-2 cells seeded was 10^5 cells/ml and the final number after reaching confluence was 2.8×10^7 cells/ml. The medium was completely removed at 24 h before adhesion assay and cells were fed with DMEM medium lacking antibiotics. The bacterial cells washed twice with phosphate buffered saline PBS (pH 7.4) and suspended at the rate of 1×10^5 cfu in 1 ml DMEM medium (without FBS and antibiotics). It was then added to different wells and incubated at 37 °C in 5% CO_2 for 2 h. The monolayer was washed five times with sterile PBS (pH 7.4). Cells from monolayer were detached by trypsinization by adding 0.25% Trypsin–EDTA solution and incubated for 15 min at room temperature. The detached cells were repeatedly aspirated to make a homogenous suspension, serially diluted with saline solution and plated on MRS Agar. The plates were incubated for 48 h at 37 °C and colonies were counted.

Results and discussion

Acid tolerance

The acid tolerance of different *L. fermentum* strains and LGG are shown in Table 1. The cultures behaved differently in various pH conditions after a different time interval. All the cultures showed an increase in one log count at pH 6.5, while the marginal reduction was observed at pH 3.5 and 3.0 for all the strains even after 3 h. At pH 2.5, 1–2 log count reduction was seen for *L. fermentum* strains except for BIF-19 and LGG. At pH 2.0, the strains NCDC-156, NCDC-606, MTCC-8711, KT-85 and LGG survived for 2 h whereas BIF-18 and BIF-19 for 1 h. However, the result at pH 2.5 is more appreciable as it is the gastric pH and the best results were shown by BIF-19 with no change in Lactobacilli count for 3 h. The results obtained are similar to those reported by other researchers in *L. fermentum* strains isolated from the human vagina and dairy products (Bao et al. 2010; Kaewnopparat et al. 2013). At low pH (less than 2), the injury due to the acid environment was revealed by the lowering of *L. fermentum* count by 2–8 log CFU/ml after incubation. In acidic conditions, protons accumulate inside the cell, which may affect the transmembrane pH gradient which is a reserve of potential energy, called as a proton-motive force. Often tolerant strains are able to regulate the homeostasis of intracellular pH by actively removing protons from the cell by the proton-translocating ATPase or by producing basic compounds (De Angelis and Gobbetti 2004).

BSH activity

The results of bile salt hydrolase activity of *L. fermentum* strains are presented in Table 2. The *L. fermentum* strains NCDC-400, MTCC 8711, BIF-18, BIF-19 and BIF-20 and the standard probiotic culture had shown intense precipitation in the two bile salts, i.e. TC and TDC, and slight precipitation for TGC. *Lactobacillus fermentum* strains C-6, C-9, NCDC-156 and KT-85 had shown slight precipitation for the three bile salts. The strains NCDC-605 and NCDC-606 did not show any precipitation of the bile salts. The results of BSH activity show the same trend of bile tolerance of these cultures in our previous study (Panicker and Behare 2014). The growth of these cultures in the presence of high bile salts concentration in the medium suggested that they showed BSH activity specific to TC, TGC and TDC. The inhibition of common intestinal bacteria has been related to the presence of free (deconjugated) bile acids rather than conjugated ones (Grill et al. 2000). *Lactobacillus rhamnosus* GG was not only able to tolerate the toxicity of these salts but also carries out BSH mediated deconjugation of TC, TGC and TDC which help in colonization of the intestine. However, the expression level of BSH is not directly proportional to resist the toxicity of conjugated bile salts. Previously, it was assumed that being a probiotic, it is necessary to show the bile salt hydrolase activity for survival in the gastrointestinal tract (GIT) (Begley et al. 2006; Tanaka et al. 1999). Now, this assumption is challenged by our group and other multiple reports using different organism (*Enterococcus faecalis, L acidophilus* NCFM, *Mesembryanthemum crystallinum, Bifidobacterium bifidum*). These studies have not detected any of the BSH protein in their data upon bile acid exposure (Giard et al. 2001; Pfeiler et al. 2007; Parijat et al. 2017; Kaur et al. 2017). Taken together, the continuous availability of the literature supports the new concept that presence of the BSH activity is not the only criteria to resist the hazardous effect of bile salt. Nevertheless, there are other mechanisms available in the bacterial system to survive in the harsh conditions and an in-depth study is required to be undertaken.

Cell surface hydrophobicity

The cell surface hydrophobicity of *L. fermentum* cultures including LGG is shown in Table 3. It was ranged from 2 to 30% and strains NCDC-400, MTCC-8711, KT-85, BIF-18, BIF-19 and BIF-20 had shown good cell surface hydrophobicity. Among the strains, *L. fermentum* MTCC-8711 exhibited the highest percentage of adhesion (25–29%) towards three hydrocarbons followed by BIF-20 (20–21%) and BIF-19 (14–21%). The microbial surface properties have been widely studied in order to understand

Table 1 Acid tolerances of *Lactobacillus fermentum* strains at different pH at 0, 1, 2 and 3 h

Cultures	Hours	Survivability of cultures in log CFU/ml (n = 2, mean ± SD)				
		pH 6.5	pH 3.5	pH 3.0	pH 2.5	pH 2.0
L. fermentum NCDC-400	0	9.20 ± 0.4	9.12 ± 0.3	8.98 ± 0.2	8.93 ± 0.4	8.67 ± 0.9
	1	9.28 ± 0.5	8.94 ± 0.4	8.61 ± 0.2	7.58 ± 0.9	0
	2	9.51 ± 0.5	8.76 ± 0.5	8.54 ± 0.4	7.17 ± 0.6	0
	3	9.63 ± 0.3	9.79 ± 0.5	7.63 ± 0.3	7.54 ± 0.4	0
L. fermentum C-6	0	9.46 ± 0.2	9.14 ± 0.9	8.96 ± 0.3	8.75 ± 0.8	8.68 ± 0.1
	1	9.51 ± 0.5	9.20 ± 0.8	8.65 ± 0.5	7.32 ± 0.2	0
	2	9.57 ± 0.8	9.17 ± 0.7	8.02 ± 0.7	7.67 ± 0.2	0
	3	9.79 ± 0.5	9.14 ± 0.3	8.08 ± 0.2	6.69 ± 0.1	0
L. fermentum C-9	0	9.38 ± 0.2	8.76 ± 0.5	8.62 ± 0.3	8.36 ± 0.1	8.32 ± 0.2
	1	9.45 ± 0.4	7.95 ± 0.4	7.84 ± 0.5	6.95 ± 0.4	0
	2	9.45 ± 0.7	8.04 ± 0.1	7.99 ± 0.6	7.16 ± 0.4	0
	3	9.49 ± 0.1	8.23 ± 0.4	8.01 ± 0.7	7.13 ± 0.7	0
L. fermentum NCDC-156	0	8.76 ± 0.3	8.80 ± 0.9	8.53 ± 0.1	8.46 ± 0.2	8.71 ± 0.7
	1	8.78 ± 0.8	8.89 ± 0.6	8.63 ± 0.3	8.39 ± 0.2	3.01 ± 0.8
	2	8.88 ± 0.5	8.90 ± 0.7	8.79 ± 0.9	8.40 ± 0.9	1.78 ± 0.1
	3	8.92 ± 0.9	8.91 ± 0.3	8.83 ± 0.2	7.37 ± 0.8	0
L. fermentum NCDC-605	0	9.41 ± 0.4	9.93 ± 0.2	9.20 ± 0.4	8.67 ± 0.2	8.60 ± 0.2
	1	9.82 ± 0.9	9.11 ± 0.5	8.54 ± 0.4	8.14 ± 0.9	0
	2	9.94 ± 0.4	8.92 ± 0.9	8.46 ± 0.2	8.05 ± 0.3	0
	3	10.04 ± 0.1	8.74 ± 0.8	7.73 ± 0.2	7.59 ± 0.1	0
L. fermentum NCDC-606	0	8.53 ± 0.1	8.94 ± 0.4	8.88 ± 0.8	8.78 ± 0.8	8.76 ± 0.3
	1	9.26 ± 0.5	7.68 ± 0.1	7.83 ± 0.8	7.62 ± 0.3	5.73 ± 0.3
	2	9.61 ± 0.2	7.51 ± 0.5	8.01 ± 0.5	7.64 ± 0.3	4.9 ± 0.8
	3	9.94 ± 0.9	7.59 ± 0.1	8.23 ± 0.4	7.74 ± 0.6	2.6 ± 0.2
L. fermentum MTCC-8711	0	8.98 ± 0.7	8.67 ± 0.2	8.81 ± 0.6	8.73 ± 0.2	8.55 ± 0.4
	1	9.10 ± 0.3	8.22 ± 0.1	7.98 ± 0.2	7.05 ± 0.9	5.49 ± 0.1
	2	9.43 ± 0.8	8.23 ± 0.5	8.01 ± 0.8	7.4 ± 0.7	5.32 ± 0.2
	3	9.46 ± 0.2	8.28 ± 0.8	8.23 ± 0.4	7.60 ± 0.2	5.2 ± 0.4
L. fermentum KT-85	0	8.97 ± 0.3	8.79 ± 0.5	8.76 ± 0.3	8.53 ± 0.1	8.60 ± 0.2
	1	9.26 ± 0.9	8.97 ± 0.8	8.80 ± 0.9	7.68 ± 0.1	5.53 ± 0.1
	2	9.68 ± 0.1	9.12 ± 0.7	8.88 ± 0.8	7.95 ± 0.9	4.45 ± 0.7
	3	9.44 ± 0.4	9.15 ± 0.2	8.94 ± 0.9	7.98 ± 0.2	3.79 ± 0.5
L. fermentum BIF-18	0	9.45 ± 0.1	9.25 ± 0.4	9.02 ± 0.1	8.99 ± 0.5	8.91 ± 0.9
	1	9.93 ± 0.9	9.65 ± 0.3	9.14 ± 0.3	8.32 ± 0.2	3.47 ± 0.7
	2	10.24 ± 0.5	9.99 ± 0.2	9.04 ± 0.1	8.71 ± 0.6	0
	3	10.43 ± 0.1	10.04 ± 0.4	8.94 ± 0.4	8.63 ± 0.3	0
L. fermentum BIF-19	0	8.71 ± 0.7	9.27 ± 0.1	9.24 ± 0.5	9.15 ± 0.6	9.12 ± 0.3
	1	10.15 ± 0.9	9.26 ± 0.7	9.24 ± 0.5	9.13 ± 0.3	3.58 ± 0.9
	2	10.19 ± 0.3	9.23 ± 0.4	9.21 ± 0.9	9.19 ± 0.3	0
	3	10.29 ± 0.5	9.22 ± 0.2	9.16 ± 0.5	9.16 ± 0.4	0
L. fermentum BIF-20	0	10.11 ± 0.3	9.18 ± 0.6	9.17 ± 0.2	9.15 ± 0.2	9.04 ± 0.1
	1	10.16 ± 0.1	9.03 ± 0.9	8.85 ± 0.1	7.82 ± 0.6	0
	2	10.17 ± 0.2	9.24 ± 0.5	8.95 ± 0.9	8.34 ± 0.2	0
	3	10.24 ± 0.5	9.66 ± 0.2	8.77 ± 0.8	8.90 ± 0.3	0

Table 1 continued

Cultures	Hours	Survivability of cultures in log CFU/ml (n = 2, mean ± SD)				
		pH 6.5	pH 3.5	pH 3.0	pH 2.5	pH 2.0
L. rhamnosus GG	0	10.19 ± 0.8	9.17 ± 0.3	9.12 ± 0.3	9.10 ± 0.3	8.96 ± 0.3
	1	10.30 ± 0.7	9.83 ± 0.8	9.19 ± 0.3	8.76 ± 0.3	5.83 ± 0.8
	2	10.91 ± 0.3	10.29 ± 0.0	9.27 ± 0.1	8.80 ± 0.3	4.70 ± 0.5
	3	11.34 ± 0.4	10.03 ± 0.7	9.76 ± 0.3	9.70 ± 0.7	3.98 ± 0.6

Presented values are means of triplicate determinations; ± indicates standard deviations from the mean

Table 2 Bile salt hydrolase activity of *Lactobacillus* cultures on different bile salts (n = 3)

Cultures	TC	TGC	TDC
L. fermentum NCDC-400	++	+	++
L. fermentum C-6	+	+	+
L. fermentum C-9	+	+	+
L. fermentum NCDC-156	+	+	+
L. fermentum NCDC-605	−	−	−
L. fermentum NCDC-606	−	−	−
L. fermentum MTCC-8711	++	+	+
L. fermentum KT-85	+	+	+
L. fermentum BIF-18	++	+	++
L. fermentum BIF-19	++	+	++
L. fermentum BIF-20	++	+	+
L. rhamnosus GG	++	+	++

− no precipitation, + slight precipitation, ++ intense precipitation

TC sodium taurocholate, *TDC* sodium taurodeoxycholate, *TGC* sodium tauroglycolate)

the interactions between bacteria and interfaces resulting in the formation of biofilms (Strevett and Chen 2003). The physical and chemical characteristics of the cell surface have been determined mainly by determining surface hydrophobicity (Geertsema-Doornbusch et al. 1993). The partitioning of cells between water and hexadecane depends on hydrophobic interactions between microorganisms and the hydrocarbon (Bouchez-Naitali et al. 2001). Previous reports by Ramos et al. (2013) reported highest hydrophobicity value of 61.0% by *L. brevis* SAU105 and lowest hydrophobicity (non-hydrophobic) by other isolates of about 1.5%.

Selection of *L. fermentum* strain for adhesion to Caco-2 cell line

The selection of strain for adhesion to Caco-2 cells was done based on the performance of the present probiotic tests as well as the results reported in our previous experiments (Panicker and Behare 2014). According to the

Table 3 Cell surface hydrophobicity of *Lactobacillus* cultures

Cultures	% cell surface hydrophobicity(n = 3, mean ± SD)		
	n-Hexadecane	n-Octane	Xylene
L. fermentum NCDC-400	11.28 ± 0.95	11.10 ± 0.73	11.16 ± 0.59
L. fermentum C-6	10.31 ± 0.88	8.45 ± 0.68	8.67 ± 0.34
L. fermentum C-9	3.39 ± 0.41	2.17 ± 0.98	3.73 ± 0.91
L. fermentum NCDC-156	4.61 ± 1.44	3.21 ± 1.12	3.89 ± 1.07
L. fermentum NCDC-605	8.09 ± 0.17	7.35 ± 0.88	8.98 ± 1.41
L. fermentum NCDC-606	1.99 ± 0.60	4.31 ± 0.37	6.20 ± 1.04
L. fermentum MTCC-8711	29.99 ± 2.97	24.66 ± 0.22	25.60 ± 1.97
L. fermentum KT-85	14.49 ± 2.59	12.77 ± 2.4	14.50 ± 1.61
L. fermentum BIF-18	17.58 ± 2.59	21.42 ± 0.62	14.39 ± 1.40
L. fermentum BIF-19	20.94 ± 2.83	14.71 ± 0.50	18.36 ± 0.78
L. fermentum BIF-20	21.41 ± 1.17	21.07 ± 0.59	19.20 ± 1.48
L. rhamnosus GG	32.14 ± 3.11	39.14 ± 2.07	28.76 ± 2.32

Presented values are means of triplicate determinations; ± indicates standard deviations from the mean

study, the best result was shown by the strain BIF-19 by tolerating the bile concentration of 1.5% for 6 h. The strain BIF-19, which shows good survivability for 3 h at pH 2.5, intense bile salt hydrolase activity against bile salts and optimum cell surface hydrophobicity (14–20%) was selected for adhesion assay.

The quantitative binding of cultures on Caco-2 cell line indicated that *L. fermentum* BIF-19 shows good adhesion property (8.78 ± 0.74) almost comparable with standard probiotic culture *L. rhamnosus* GG (9.98 ± 0.36). The studies on the adhesion of urogenital lactobacilli to intestinal epithelial cells concluded that adhesion to epithelial cells was correlated with bacterial hydrophilicity, whereas an extracellular protein and a trypsin-insensitive cell wall factor were involved in adherence to Caco-2 cells (Sriphannam et al. 2012). According to the study by Greene and Klaenhammer (1994) on human intestinal isolates, *L. acidophilus* BG2FO4 and NCFM/N2 and *L. gasseri* ADH, adhered to Caco-2 cells at levels which were 0.5–1 log unit higher than the level of adherence obtained with a dairy isolate *L. delbrueckii* subsp. *bulgaricus* 1489. Another study by Ramos et al. (2013) on the isolates *L. plantarum* SAU96 and CH3 showed higher percentages (1.8 and 1.6% of adhesion, respectively) of adhesion to Caco-2 cells compared to the positive control *L. rhamnosus* GG (1.5%). The isolates *L. plantarum* CH41, *L. brevis* FFC199 and *L. fermentum* CH58 showed moderate (1.1, 0.9 and 0.8%, respectively) adhesion ability while the *L. brevis* SAU105 (0.3%) isolates showed a lower percentage of adhesion to Caco-2 cells. This gives a contradiction to our result of cell adhesion using Caco-2 cell lines by LGG. But the study by Dimitrov et al. (2014) and Singh et al. (2017) gives the cell adhesion percentage of 9.7 ± 3.3 which correlates well with our results. This gives a clear indication that the result directly depends upon the initial number of Caco-2 cells and the method used for analysis.

Conclusion

All the analysed *L. fermentum* strains were tolerant to pH 2.5. By showing bile salt hydrolase activity and good cell surface hydrophobicity towards the hydrocarbons and Caco-2 cell line, BIF-19 was able to tolerate bile stress and adhere to the intestinal epithelial cell surface. The human faecal origin strain BIF-19 demonstrated the best preliminary probiotic properties for the tests under study, but necessitate in-depth evaluation for other important probiotic features such as the production of antimicrobial compounds, inhibition/exclusion of pathogens, anti-inflammatory properties etc. The prerequisite of probiotic interest should be assessed in vivo too, in order to ascertain the real capacity of the strains to survive transit through the gastrointestinal tract.

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