Arch Microbiol (2015) 197:155–164
DOI 10.1007/s00203-014-1034-7

ORIGINAL PAPER

Adhesion of indigenous *Lactobacillus plantarum* to gut extracellular matrix and its physicochemical characterization

Ashok Kumar Yadav · Ashish Tyagi · Ashwani Kumar · Asha Chandola Saklani · Sunita Grover · Virender Kumar Batish

Received: 18 May 2014 / Revised: 24 August 2014 / Accepted: 26 August 2014 / Published online: 12 September 2014

Abstract Adhesion to the human intestinal epithelial cell is considered as one of the important selection criteria of lactobacilli for probiotic attributes. Sixteen *Lactobacillus plantarum* strains from human origins were subjected for adhesion to extracellular matrix (ECM) components, and their physiochemical characterization, incubation time course and effect of different pH on bacterial adhesion in vitro were studied. Four strains showed significant binding to both fibronectin and mucin. After pretreatment with pepsin and trypsin, the bacterial adhesion to ECM reduced to the level of 50 % and with lysozyme significantly decreased by 65–70 %. Treatment with LiCl also strongly inhibited (90 %) the bacterial adhesion to ECM. Tested strains showed highest binding efficacy at time course of 120 and 180 min. Additionally, the binding of Lp91 to ECM was highest at pH 6 (155 ± 2.90 CFU/well). This study proved that surface layer components are proteinaceous in nature, which contributed in adhesion of lactobacillus strains. Further, the study can provide a better platform for introduction of new indigenous probiotic strains having strong adhesion potential for future use.

Keywords *Lactobacillus plantarum* · Adhesion · Extracellular matrix · Physicochemical characterization

Communicated by Erko Stackebrandt.

A. K. Yadav (✉)
Department of Microbiology and Immunology, National Institute of Nutrition, Hyderabad, India
e-mail: ashok.ndri@gmail.com

A. Tyagi · S. Grover · V. K. Batish (✉)
Molecular Biology Unit, Dairy Microbiology Division, National Dairy Research Institute, Karnal 132001, Haryana, India
e-mail: vkbatish@gmail.com

A. Kumar
Department of Biotechnology, Seth Jai Parkash Mukand Lal Institute of Engineering and Technology, Radaur, Yamuna Nagar 135133, Haryana, India
e-mail: ashwanindri@rediffmail.com

A. C. Saklani
School of Life Sciences, Appejay Stya University, Gurgaon, Haryana, India

Introduction

Among lactic acid bacteria (LAB), the genus *Lactobacilli* are commonly used as probiotic organisms, which help to maintain a balanced intestinal microbiota, detoxifying colonic toxins, lowering serum cholesterol levels (Kumar et al. 2011; Grover et al. 2012), promoting lactose tolerance, producing metabolites crucial to the function of intestinal epithelial cells (Szilagyi et al. 2010), excluding pathogens and assisting to keep the gut homeostasis by influencing the mucosal immune system (Kumar et al. 2011; Duary et al. 2012a; Hardy et al. 2013; Yadav et al. 2013). Colonization of a probiotic strain on the mucosal surface is undoubtedly a primary prerogative for stable and successive exertion of these beneficial effects in the gut. Successful colonization is a direct consequence of effective bacterial adhesion to the gut components primarily with EMC (Styriak et al. 2003). Bacterial adhesion is a complex process initially based on non-specific physical interactions between two surfaces, which then allow specific interactions between adhesin proteins and their receptors (Pérez et al. 1998; Letourneau et al. 2011; Turroni et al. 2013). Additionally, several other factors viz. retention time in the intestine, specific physicochemical properties and adhesion sites have been observed and known to

influence colonization of probiotic to gut epithelial cells (Schillinger et al. 2005; Botes et al. 2008; Rodríguez et al. 2012). Epithelial cells of gastrointestinal tract are covered with a layer of mucus that protects from damage and pathogens (Tuomola et al. 1999a). Keeping in mind these important factors and composition of the aforementioned mucus layer, several models have been developed to assess the adhesive properties of *lactobacilli*, binding to tissue culture cells (Tuomola et al. 1999b), resected colonic tissue (Ouwehand et al. 2001; Vesterlund et al. 2005), intestinal mucus (Ouwehand et al. 2001) and extracellular matrix (ECM) proteins (Lorca et al. 2002; Styriak et al. 2003; De Leeuw et al. 2006). Bacterial adhesion had been performed using fibronectin, laminin and collagens (types I and IV) (Antikainen et al. 2002; Styriak et al. 2003, Yadav et al. 2013). The culture cell lines HT-29, Caco-2, EA-hy926 and intestine 407 (ATCCCCL6) from human colon and intestine have been used (Adlerberth et al. 1996; Hynonen et al. 2002).

These studies strongly reflect that strains of LAB such as *L. rhamnosus* GG and *L. johnsonii* La1 have high adherence potential vis a vis human colonic Caco-2 cell line, HT-29 and EA-hy926 cell lines among others (Munoz and Monedero 2011; Duary et al. 2011; Maria Hidalgo et al. 2012). Although studies highlighting the potent role of adhesion in bacterial capability to bestow beneficial effects have been in plenty in the last decade or two, the underlying mechanisms of adhesion are unclear. At the same time, several studies have demonstrated the involvement of bacterial surface proteins using protease pretreatments of the bacterial cells (Tuomola et al. 1999a; Lorca et al. 2002; Caballero-Franco et al. 2007) or by purified cell surface proteins that adhere to a matrix (Roos et al. 1996; Sillanpaa et al. 2000, Yadav et al. 2013). A previous study demonstrated the strain-dependent and mannose-specific adhesion of *Lactobacillus plantarum* strains to HT-29 cells (Adlerberth et al. 1996). Characterization of several LAB adhesion proteins such as the mucus-binding protein (Mub) (Roos and Jonsson 2002), mucus-adhesion-promoting protein (MapA) (Miyoshi et al. 2006), collagen binding protein (cbp) (Yadav et al. 2013) elongation factor Tu (EF-Tu) (Granato et al. 2004) and GroEL (Bergonzelli et al. 2006), surface layer proteins (Slp) (Boot et al. 1993, Vidgren et al. 1992) and aggregation-promoting factors (apf1 and apf2) (Jankovic et al. 2003; Ventura et al. 2002) have also helped in the studies pertaining to better understanding of involved mechanisms.

Our previous studies have shown that indigenous Lactobacillus strains viz. Lp9, Lp77 and Lp91 demonstrate excellent probiotic efficacy and potential in terms of effective tolerance to low pH and high bile salt concentrations (Kumar et al. 2011; Duary et al. 2012b; Kumar et al. 2012), high cell surface hydrophobicity (Kaushik et al. 2009; Duary et al. 2010), adhesion ability to Caco-2 cell line and immunomodulatory effects in gut (Duary et al. 2011, 2012a). These studies have also highlighted that these particular strains have high adhesion to human type-1 collagen and demonstrated anti-adhesion potential of purified cbp against gut pathogens (Yadav et al. 2013).

Examination of the binding ability of selected probiotic strains to other ECM components will help us to better understand the underlying mechanism and confirm the strong adhesion capability of selected probiotic strains. In the current study, strains of *L. plantarum* from human origins were employed for in vitro adhesion to ECM components, which have not been studied previously. We also investigated the effect of several enzymes, chemicals, time course and pH on adhesion properties of selected strains.

Materials and methods

Bacterial strains, media and growth conditions

The *L. plantarum* strains employed in this study are listed in Table 1. Strains were isolated from healthy human fecal samples. The strain *L. plantarum* NCDO5276 (Crittenden et al. 2002) (received from Molecular Biology Unit, National Dairy Research Institute, Karnal, Haryana, India) was used as a reference culture. Isolates were cultured in MRS broth (deMan, Rogosa and Sharp broth; HiMedia, Mumbai, India) at 37 °C. All the ECM components (mucin, collagen and fibronectin) were purchased from Sigma Aldrich.

In vitro binding of probiotic lactobacilli to extracellular matrix components

Screening of ECM binding of *L. plantarum* strains were performed using in vitro adhesion assay (Tallon et al. 2006; Diego et al. 2009) with a few modifications. Briefly, *L. plantarum* strains were assayed for binding to different ECM substrates immobilized on 96-well microtiter plates. Plates were incubated with the ECM substrate mucin (porcine) and fibronectin (human plasma) at a concentration of 500 µg/ml and 50 µg/ml in 50 mM phosphate buffer pH 7.0, respectively, and subsequently incubated overnight at 4 °C. After immobilization, wells were washed three times with PBS and blocked with 2 % (w/v) bovine serum albumin (BSA) (Sigma) solution for 4 h at 4 °C. A minimum of three replicates were used twice to estimate the adhesion of the strains. Fresh bacterial culture (100 µl of 10^8 CFU/ml) of individual strain was added, and plates were incubated for 2 h at 37 °C. Repeated washing was followed with treatment of wells with 200 µl of a 0.05 % (v/v) triton X-100 solution to remove the bound bacteria and 100 µl

Table 1 Adhesion of *L. plantarum* strains on immobilized ECM (fibronectin and mucin) substrates

Strain	Sources of isolation	Adhesion on Fibronectin (CFU/well)	Adhesion on Mucin (CFU/well)
Lp5276	SMS, Australia	172.33	146.66
Lp9	Human fecal	135.33	128.66
Lp40	Human fecal	103.00	66.00
Lp41	Human fecal	126.66	48.00
Lp44	Human fecal	83.66	45.33
Lp71	Human fecal	116.33	122.33
Lp72	Human fecal	155.66	125.66
Lp75	Human fecal	110.33	83.66
Lp76	Human fecal	124.66	68.00
Lp77	Human fecal	133.66	110.66
Lp78	Human fecal	76.33	85.00
Lp80	Human fecal	64.33	44.33
Lp90	Human fecal	116.33	86.33
Lp91	Human fecal	184.33	138.00
Lp95	Human fecal	59.66	36.33
Lp121	Human fecal	113.66	42.33
Lp122	Human fecal	112.33	52.66

was aspirated from each well. This was diluted in sterile PBS and plated on MRS agar plates. After 18–24 h of incubation at 37 °C, bacterial colonies were counted from each plate.

Real-time quantitative PCR analysis of the mub and fbp protein

Lactobacillus cells were grown early to mid-exponential phase, OD_{600} ~0.5–1.0 in MRS, and total RNA was isolated from low, moderate, high ECM-binding lactobacillus cells using the standard Trizol method to study relationship between level of *mub* and *fbp* gene expression and adhesion capacity. qRT-PCR was carried out using LightCycler 480 SYBR Green I Master technology (Roche Diagnostics, Mannheim, Germany) with gene-specific primer pairs MubRT_F/MubRT_R and FbpTR_F/FbpRT_R (Table 2). 16S rDNA was used as internal control gene.

Table 2 Primers used in this study

Primer set	Sequence (5′–3′)	Amplicons size
MubRT__F	CCGTTACTACGACGGATGGG	209 bp
MubRT_R	CATTGCATAAGTGCCGACCG	
FBPRT_F	CGCAAAAGTGCTGCTCGTTA	226 bp
FBPRT_R	AGCAGTGGTGTAATCCGCTC	

Pretreatment of bacteria with enzymes and chemicals

Four strains (Lp9, Lp71, Lp72 and Lp91) as well as standard strain (NCDO5276) were subjected to several pretreatments in order to investigate the effect of different enzymes and chemicals on their adhesion to ECM substrates (mucin, collagen and fibronectin). For this study, the overnight-grown bacterial cells were washed twice in sterile PBS (pH 6.0) (Tuomola et al. 2000). Briefly, the bacterial suspension (10^8 CFU/ml) was incubated with different enzymes trypsin (2 mg/ml), pepsin (2 mg/ml) and lysozyme (1 mg/ml) for 1 h at 37 °C. Cell suspension was mixed in equal volume of 2 M LiCl solution followed with incubation at 4 °C for 30 min under gentle agitation. Pretreated bacterial culture (100 µl of 10^8 CFU/ml) of individual strain added on microtiter plates (precoated with mucin, collagen and fibronectin) were incubated for 2 h at 37 °C.

Bacterial adhesion at different incubation time on extracellular matrix

The binding ability of selected probiotic strains to ECM (collagen) immobilized on microtiter plate was determined at different time intervals. Selected strains (Lp91 and NCDO5276) were assayed for binding to ECM substrates immobilized on 96-well microtiter plates. Changes in adhesion capability of probiotic strains with respect to increasing time (15, 30, 60, 90, 120 and 180 min) were examined to confirm for binding efficiency.

Adhesion of bacteria at different pH

Adhesion ability of probiotic strains were assessed at different pH levels 5.5, 6.0, 6.5, and 7.0 using PBS buffer. One molar solution of HCl and NaOH was used to adjust the pH level of PBS buffer. Fresh overnight-grown culture

of lactobacillus was centrifuged at 3,000*g* for 10 min, discarded the supernatant, and pellet was dissolved in PBS (10^8 CFU/ml) at different pH and then mixed using vortex.

Result and discussion

Adhesion of *L. plantarum* to extracellular matrix substrates

Colonization of a probiotic strains on the mucosal surface, i.e., to the ECM components is certainly a perquisite for stable and successive exertion of beneficial effects in the gut. Probiotic strains mimic the same mechanism as used by the pathogens, and hence, the binding ability to collagen, as demonstrated in our previous study (Yadav et al. 2013), is quintessential in determining the ability of a putative probiotic strain to colonize in the gut environment. ECM components other than collagen have also been observed to be actively interacting with bacterial population in gut (Mackenzie et al. 2010), and examination of the binding ability of selected probiotic strains to these ECM components such as mucin, collagen, fibronectin, albumin, vitronectin and also the analysis of binding ability of selected probiotic strains will help us to better understand the underlying mechanism and confirm the strong adhesion capability of selected probiotic strains. The present study investigates and analyzes the adhesion ability of indigenous probiotic lactobacilli isolates on distinct ECM of human gut.

Our previous study revealed a correlation between cell surface hydrophobicity and binding capacity of a putative probiotic *L. plantarum* strain (identified at genus and species level) on human type-1 collagen. Collagen, mucin and fibronectin being major components of the ECM are common targets for bacterial attachment, including *lactobacilli* (Velez et al. 2007). Hence, binding of probiotic *Lactobacilli* strains with mucin and fibronectin immobilized on microtiter well plates was determined in this study.

All the test cultures were adhered to immobilized mucin and fibronectin at different level (Table 1). Selected strains demonstrated better binding with fibronectin as compared to mucin. Thirteen strains showed significant binding with fibronectin, while four strains with mucin with respect to reference strain NCDO5276. Six strains viz. Lp9, 41, 72, 76, 77 and 91 have significant adhesion to fibronectin in the range of 124–184 CFU/well as compared to reference strains NCDO5276 (146.66 CFU/well). Lp91 (184.33 CFU/well) showed 6 % higher adhesion as compared to reference strain NCDO5276 (172.33 CFU/well), whereas four strains Lp9, 71, 72 and 91 also showed significant binding with mucin (122.33–138.00 CFU/well) compared with reference strain NCDO5276 (146.66 CFU/well). The results show that the four test strains (Lp9, Lp71, Lp72 and Lp91) and reference strain NCDO5276 have comparable and even better adhesion with both substrates in some instances (Fig. 1). Our results also indicated that Lp91 had higher binding affinity toward fibronectin (184.33 CFU/well) and collagen (177.66 ± 11.50 CFU/well) as compared to mucin (138.0 CFU/well), which is a deviation from the normal pattern, shown by other strains. It is clear from these findings that binding affinity of a *lactobacillus* isolate with different ECM components is an independent event, i.e., a *lactobacillus* strain does not necessarily bind to all the ECM components in a fixed pattern. Nevertheless, a strain with better probiotic potential effectively shows strong binding with all the ECM components as was the case with Lp91 in the present study.

As demonstrated by other studies (Elli et al. 2006; Balgir et al. 2013), bacterial strains that can remain viable after passage through the human stomach may only remain in the small intestine for a several hours. It seems feasible that if a strain can adhere to the intestinal wall, residence

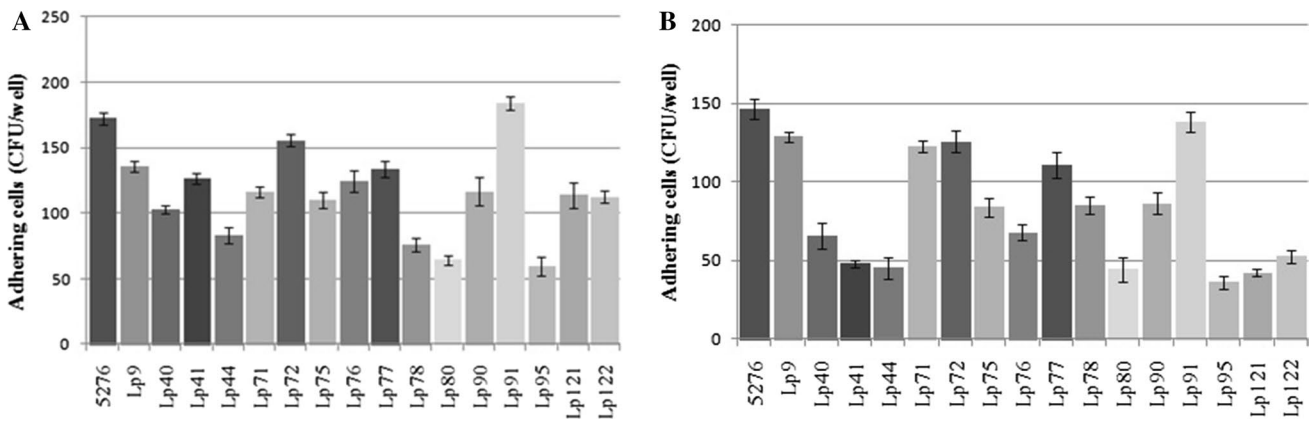

Fig. 1 Adhesion assay of different *L. plantarum* strains. Binding of *L. plantarum* strains to **a** fibronectin, **b** mucin immobilized on microtitre plates. *Error bars* represents standard error of mean

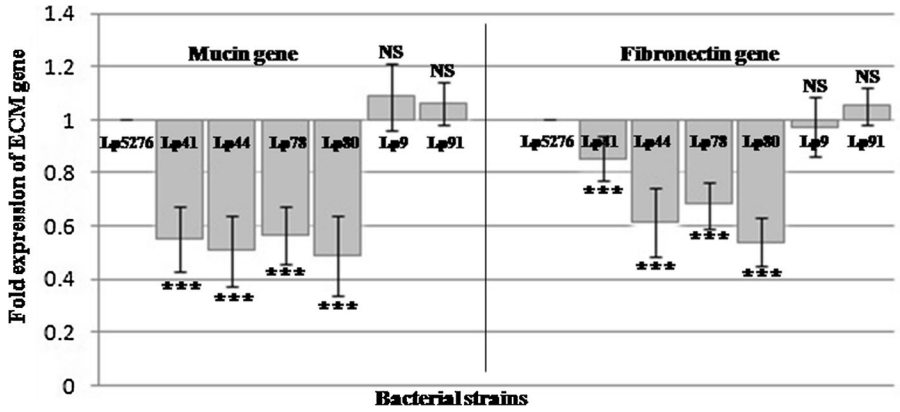

Fig. 2 Relative expression of mub and fbp gene in selected *L. plantarum* strains as compared to Lp5276 (NCDO5276). *Error bars* represents standard deviations (±SD). *NS* non-significance, ***Significance

time could be extended to allow the bacterial cell sufficient time to multiply and, if possible, to colonize. Significant binding efficiency of some strains used in this study make them good candidate in this regard. Strain-specific affinity toward ECM components has also been observed in studies such as by Diego et al. (2009), *L. casei* isolated from different origin showed intermediate binding with some ECM components, while other showed lowest binding ability to the same ECM components (Lorca et al. 2002; Styriak et al. 2003; Velez et al. 2007).

To further characterize the possible link between variation in gene expression and binding ability to mucin and fibronectin among selected lactobacillus strains and to explore correlation between adhesion capability and transcriptional level expression of mub and fbp gene, quantitative real-time PCR (qRT-PCR) analysis was performed. On the basis of adhesion capability to immobilized ECM, six bacterial strains (Lp41, Lp44, Lp78, Lp80, Lp9 and Lp91) as well as reference strain (NCDO5276) were selected. Relative expression of mub and fbp gene in strain Lp41, Lp44, Lp78 and Lp80 were significantly different (<0.05), while strains Lp9 and Lp91 demonstrated nonsignificant difference (>0.05) with reference strains NCDO5276 used as control. Expression of mub gene was up-regulated approximately 1.08, and 1.06-fold in strains Lp9 and Lp91, respectively, while expression of fbp gene was up-regulated approximately 1.05-fold only in strain Lp91 as compared to reference strain (NCDO5276) (Fig. 2). These results are in agreement with our previous study which showed that the Cbp transcript level was significantly up-regulated in three strains Lp9, Lp72 and Lp91, which showed strong adhesion to immobilized collagen (Yadav et al. 2013). The results are also in corroboration with studies which have reported that the level of mub gene expression are significantly associated with in vitro adhesion ability of lactobacillus to immobilized mucus and collagen (Ramiah et al. 2009; MacKenzie et al. 2010; Yadav et al. 2013). Another study reported successful display of mature adhesin on the surface of probiotics lactobacilli as a crucial factor for binding to ECM components (Castaldo et al. 2009; Sun et al. 2012; Duary et al. 2012b).

Effect of enzymes and biochemical on bacterial adhesion

Adhesion properties depend on a variety of factors including non-specific adhesion determined by electrostatic or hydrophobic forces and specific binding dependent on binding to intestinal mucus and ECM components (Lorca et al. 2002; Styriak et al. 2003). Bacterial pretreatment with enzymes and chemicals affect the other Slp, which are also major factors in specific adhesion.

The selected probiotic strains (Lp9, Lp71, Lp72 and Lp91) along with reference strain (NCDO5276) were further subjected to enzymatic and biochemical treatments to explore the mechanism involved in adhesion. The treatment with LiCl strongly inhibited (90 %) the adhesion of test cultures to all ECM substrates. Pretreatment with lysozyme also significantly decreased the adhesion to the level of 65–70 %, whereas after pretreatment with pepsin and trypsin, the probiotic adhesion decreased to a level of 50 % as can be inferred from Table 3 and Fig. 3.

Previous reports have also indicated significant effects of enzymatic and chemical treatments on binding capacity of treated cells with mucus proteins. However, the effect was more prominent in case of chemical treatment (Tallon et al. 2006). Similar results were also reported in a study by Mukai et al. (1996) who observed that pretreatment with trypsin resulted in only a slight decrease in adhesion to collagen and mucin, while pretreatment with GnHCl or urea resulted in 90 % reduction in adhesion. Conversely, a study by Tallon et al. (2006) reported that adhesion was inhibited by trypsin treatment in some of the strains, but the same was not influenced by LiCl treatment. It was suggested by the investigators of the study that several proteins involved in adhesion differed in their physicochemical properties such as hydrophobicity or accessibility. The results obtained in the present study, after an incubation of the bacterial cells in a solution of proteinases (trypsin,

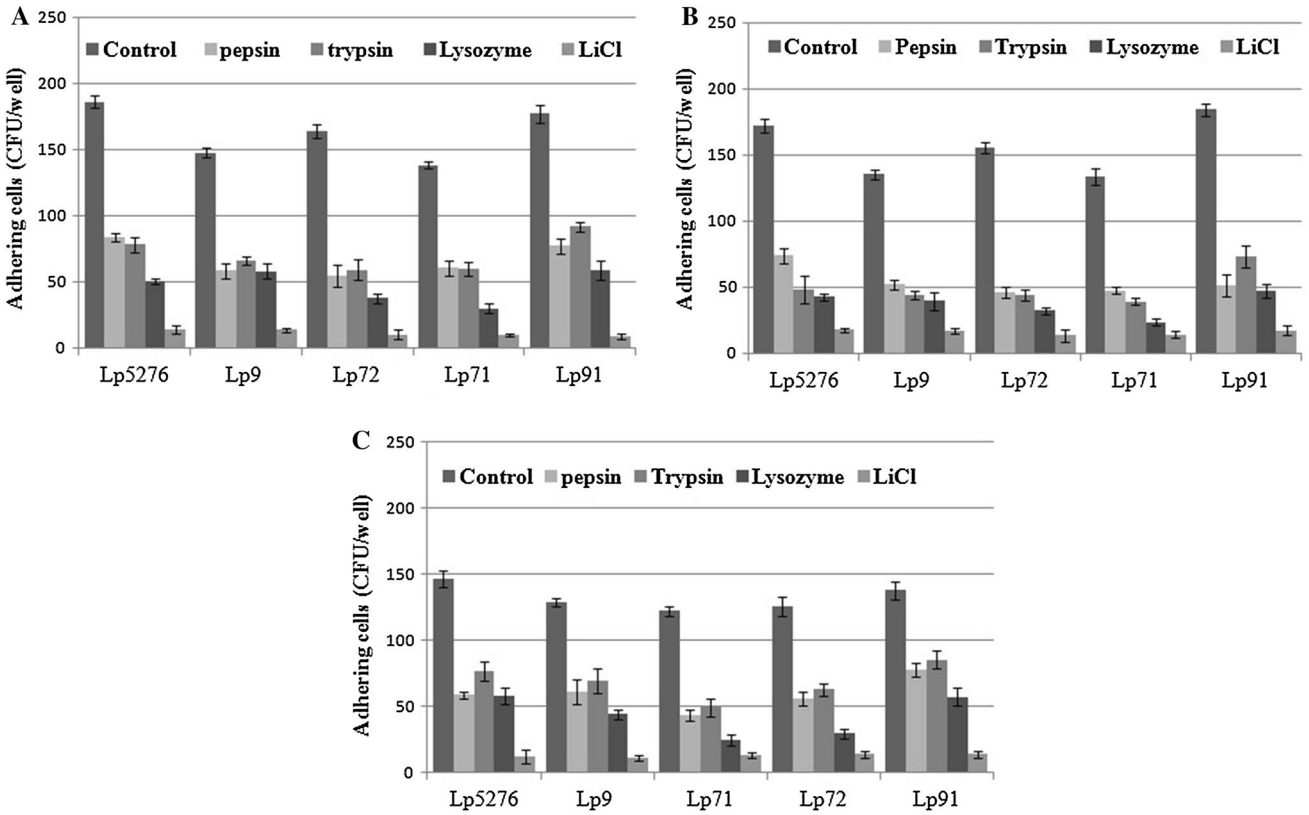

Fig. 3 Effect of enzymes and chemical treatment on the binding potential of selected *L. plantarum* strains cells resuspended in PBS were subjected to digestion with pepsin (2 mg/ml), trypsin (2 mg/ml), lysozyme (1 mg/ml) and Lithium chloride (2 M) for 1 h at 37 °C and used in binding studies to **a** collagen, **b** fibronectin and **c** mucin. *Error bars* represent standard error of mean

Table 3 Adhesion properties of indigenous isolates to ECM i.e. collagen, fibronectin and mucin

Strains	Control	Pepsin	Trypsin	Lysozyme	LiCl
a. Adhesion on collagen (CFU/well)					
Lp5276	186.33 ± 4.63	84.00 ± 3.6	78.66 ± 5.78	50.66 ± 2.4	14.33 ± 3.48
Lp9	148.00 ± 3.78	58.66 ± 5.48	66.33 ± 3.38	58.33 ± 5.78	13.66 ± 1.76
Lp72	164.33 ± 5.48	54.66 ± 8.68	59.33 ± 7.83	38.00 ± 3.78	10.33 ± 3.75
Lp71	138.66 ± 2.4	60.66 ± 6.11	60.00 ± 5.5	30.33 ± 3.92	10.00 ± 1.52
Lp91	177.66 ± 6.64	77.66 ± 5.81	92.00 ± 3.46	59.00 ± 6.8	8.66 ± 2.02
b. Adhesion on fibronectin (CFU/well)					
Lp5276	172.33 ± 4.97	74.00 ± 6.02	48.00 ± 10.39	42.66 ± 2.6	17.55 ± 1.45
Lp9	135.33 ± 4.09	52.00 ± 3.51	44.00 ± 3.51	39.66 ± 6.93	17.00 ± 2.3
Lp72	155.66 ± 4.33	46.33 ± 4.05	44.33 ± 4.05	32.33 ± 2.6	13.33 ± 4.7
Lp71	133.66 ± 5.92	47.33 ± 2.72	39.00 ± 2.72	23.66 ± 2.96	14.33 ± 2.4
Lp91	184.33 ± 4.97	51.00 ± 8.32	73.33 ± 8.32	47.66 ± 5.23	17.33 ± 3.92
c. Adhesion on mucin (CFU/well)					
Lp5276	146.66 ± 6.48	58.66 ± 2.9	76.66 ± 6.69	58.00 ± 6.96	12.33 ± 5.36
Lp9	128.66 ± 3.17	60.66 ± 9.33	69.33 ± 4.04	44.00 ± 9.2	10.66 ± 2.02
Lp71	122.33 ± 3.71	43.66 ± 4.33	49.33 ± 4.05	24.66 ± 7.05	13.33 ± 2.02
Lp72	125.66 ± 6.88	55.66 ± 5.2	62.66 ± 3.75	29.66 ± 5.04	13.66 ± 2.6
Lp91	138.00 ± 6.65	78.00 ± 5.23	85.33 ± 6.38	57.33 ± 6.98	13.66 ± 2.6

Table 4 Adhesion of *L. plantarum* strains to immobilized collagen at different time intervals

Strains	Bacterial counts (CFU/well)					
	15 min	30 min	60 min	90 min	120 min	180 min
NCDO5276	5.33 ± 1.76	13.00 ± 2.08	41.66 ± 2.02	75.00 ± 6.24	159.00 ± 11.06	162.00 ± 11.71
Lp9	4.66 ± 1.76	12.33 ± 1.76	33.00 ± 3.21	63.66 ± 2.60	122.66 ± 12.25	129.00 ± 16.09
Lp71	3.33 ± 0.88	11.33 ± 1.45	31.00 ± 4.61	53.33 ± 2.60	120.33 ± 7.51	148.66 ± 16.49
Lp72	6.66 ± 1.20	10.33 ± 2.60	32.00 ± 3.51	51.00 ± 4.72	148.33 ± 10.68	158.66 ± 15.5
Lp91	2.51 ± 2.18	15.33 ± 1.45	30.33 ± 3.28	58.33 ± 3.75	155.00 ± 9.40	159.33 ± 13.95

Table 5 Adhesion of selected *L. plantarum* strain to immobilized collagen at different pH

Strain	pH 5.5	pH 6.0	pH 6.5	pH 7.0
Lp5276	139.66 ± 4.09	159 ± 6.24	85 ± 6.80	56 ± 4.72
Lp91	127.66 ± 2.60	155 ± 2.90	64.33 ± 6.88	45 ± 4.35

Pepsin and Lysozyme), strongly suggest that the bacterial components involved in their adhesion were proteins and/or glycoproteins and use of Lithium chloride that is commonly used to extract bacterial Slp, thus drastically reduced the adhesion capability of a probiotic strain.

Outcome of few other studies have also demonstrated the proteinaceous nature of *Lactobacillus* strain being responsible for their adhesion capability (Greene and Klaenhammer 1994; Tuomola et al. 2001; Ouwehand et al. 2001). This may well be the reason for loss in adhesion capability of selected putative probiotic strains examined in this study, when treated with proteinases.

Bacterial adhesion at different time and pH

An extended transit period through the gut aids better colonization thereby resulting into most optimal expression of health-promoting functions by a putative probiotic strain. Therefore, a possible influence of extended exposure of probiotic strains to the ECM component, on their adhesion capability, was investigated in this study.

Changes in adhesion capability of these isolates with respect to increasing time (15, 30, 60, 90, 120 and 180 min) indicated that almost all the isolates demonstrated high level of binding or rate of adhesion at 120 and 180 min (Table 4).

The adherence of *L. plantarum* to ECM was assessed quantitatively at different pH level by count of CFU of adhered bacteria plated on MRS agar plate (Fig. 4b). Results from three independent experiments performed in triplicates are shown in Table 5. The adhesions of Lp91 and NCDO5276 were approximately similar on human type-1 collagen but varied at different pH level. Lp91 (155 CFU/well) and NCDO5276 (159 CFU/well) showed highest adhesion at pH level 6.0, while adhesion of Lp91 (127.66 ± 2.60) and NCDO5276 (139.66 ± 4.09) was decreased at pH level 5.5. When pH level was increased then adhesion of both Lp91 (64.33 ± 6.88) and NCDO5276 (85.0 ± 6.80) at pH 6.5; and Lp91 (45.0 ± 4.35) and NCDO5276 (56.0 ± 4.72) at pH 7.0 was abruptly decreased (Table 5; Fig. 4b). Considering the fact that it is imperative for a bacterium to remain in contact with ECM for a longer period of time to confer its beneficial effects, the same should hold true in case of binding efficiency with

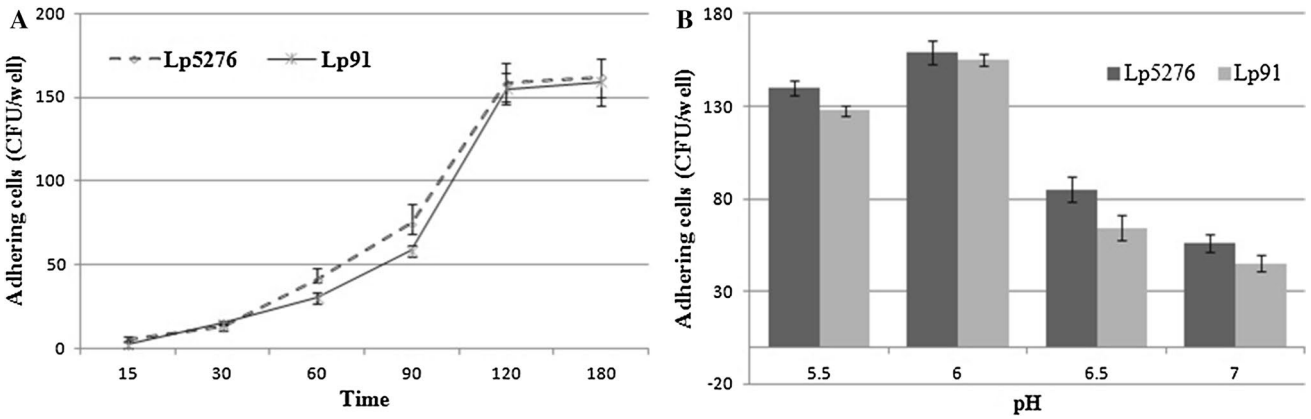

Fig. 4 Effect of time course (**a**) and pH (**b**) on adhesion ability of *L. plantarum* strain to immobilized collagen

increasing time period. The results of this study are in close agreement with that of a study by Ali et al. (2009) in which *bifidobacterium* strains were investigated for their adhesion on HT-29 cell lines at different pH and time interval. Highest adhesion was recorded at pH 5.6 and 120 min, thereby substantiating the validity of our result in this regard. In our study, highest adhesion was shown at pH 6.0 and 120 and 180 min.

Conclusion

The results presented here illustrate adhesion capability to be a strain-specific attribute. However, the same study also provides a molecular insight reflecting a significant correlation between this adhesion capability and regulation at transcript level. Lp9, Lp72, Lp77 and Lp91 demonstrated high binding capability to ECM proteins and also upregulation of mub as well as fbp gene. Effect of proteases on adhesion potential of lactobacilli strains suggest that the mechanism of attachment involves interacting entities, which are proteinaceous in nature. The study also underscores the fact that the role of distinct components (such as mucin, collagen, fibronectin among several others) of an ECM could not be understood and studied in isolation but as an aggregate.

The success of lactobacilli to achieve the desired probiotic effects including maintaining healthy intestinal ecosystem would largely depend on their ability to survive the gastrointestinal stressful conditions along with the given antibiotic. Consequently, the selection of a *Lactobacillus* strain for a probiotic application as prophylactic agent must take into account changes in its susceptibility to antibiotics due to various stressors encountered in the gastrointestinal tract.

Pretreated *L. plantarum* strains with enzymes (pepsin, Trypsin and lysozyme) and chemical (Lithium chloride) significantly decreased binding capability to ECM proteins. Optimal time was 120 min and pH 6.0 for lactobacillus strain for binding to ECM proteins. Surface protein responsible for adhesion should be investigated in more detail. An indigenous strain can serve as the potential probiotic candidate, which should be further subjected to in vivo studies in order to explore its novel health-promoting functions due to better colonization in the gut.

Acknowledgments The authors greatly appreciate the financial support received from Department of Biotechnology, Govt. of India, India.

Conflict of interest None of the authors have a conflict of interest for the publication of the manuscript.

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