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ORIGINAL ARTICLE

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Characterization and in vitro probiotic evaluation of lactic acid bacteria isolated from *idli* batter

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Abstract An Indian traditional fermented food, *idli* batter, was used as a source for isolation of lactic acid bacteria (LAB). A total of 15 LAB strains were isolated on the basis of their Gram nature and catalase activity. Of these, one lactobacilli strain and one lactococci strain which showed antimicrobial activity were identified using biochemical characterization, sugar utilization and molecular sequencing. The microbes, labeled as IB-1 (*Lactobacillus plantarum*) and IB-2 (*Lactococcus lactis*) were tested for their in vitro tolerance to bile salts, resistance to low pH values and acidifying activity. Both the strains showed good viability (IB1- 58.11%; IB2- 60.84%) when exposed to high bile salt concentration (2%) and acidic pH of ≤pH 3.0 (IB1- 88.13%; IB2- 89.85%). Lactic acid (IB1- 181.93 mM; IB2- 154.44 mM), biomass production (IB1- 0.65; IB2- 0.58 g/l) after 54 h as well as qualitative estimation of β-galactosidase and vitamin B_{12} production were also studied to check their suitability as an industrially important strain for production of important biomolecules.

Keywords *Idli* batter · Probiotic · Lactic acid bacteria · Bile tolerance

Introduction

In recent decades, extensive research has been carried out on isolation and screening of microorganisms from traditional fermented foods due to their eco-friendly and genetically sturdy nature. Lactic acid bacteria (LAB) and yeasts play an important role in numerous natural food fermentations such as curd, cheese, pickles and various other traditional foods. Furthermore, they are closely associated with the human environment. LAB associated with fermented foods include species of the genera *Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella* (Stiles and Holzapfel 1997). These organisms have also gained popularity as probiotics (Rivera-Espinoza and Gallardo-Navarro 2010). Probiotics are live micro-organisms which, when taken in adequate amounts beneficially affect the host by improving the intestinal microbial balance (Fuller 1989). Among these microorganisms, LAB is regarded as a major group of probiotic bacteria (Collins et al. 1998).

A strain suitable as a probiotic should satisfy various criteria such as ability to resist acidic pH of stomach and bile salts produced at the opening of the intestinal tract, retain viability, and adhere to mucosal surfaces (Goldin and Gorbach 1992, Holzapfel et al. 1998). In addition, these strains should be non pathogenic and have a "generally regarded as safe" (GRAS) status. They should be able to grow easily and have a moderately good survival period. Their health benefits should also be well documented (Stanton et al. 2003). Generally probiotic cultures are used to reinstate the body's naturally occurring gut flora after a course of antibiotics. Probiotics are used in controlling blood pressure and cholesterol levels, preventing gastrointestinal infection by pathogenic bacteria, treatment of diarrhoea and urogenital diseases (Reid 1999). Studies have also shown that probiotics strengthen the immune system to fight against allergies, stress, excessive alcohol intake and other diseases (Nichols 2007, Sanders 2003). Probiotics are additionally being scrutinized as live delivery medium to

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carry vaccines, antimicrobials or enzymes to targeted locations on the GI tract or mucosal surfaces. Isolated LAB's are also used for production of lactic acid and polylactic acid (PLA). Fermentation is preferred for production of lactic acid due to favourable economics of production. PLA is a biodegradable material that has been used for orthopaedic implants, surgical sutures, disposable products and drug delivery systems (Adnan and Tan 2007).

Probiotic products have shown a rapid growth in the global food market due to increased consumer awareness on health and their demand for healthy foods. The cultures belonging to the genera *Bifidobacterium*, *Lactobacillus, Lactococcuus* and *Saccharomyces*, are used in many products ranging from fermented dairy products and infant food to pharmaceutical preparations.

A large number of LAB, isolated from various fermented food systems in different parts of the world have been studied for their probiotic potential and ability to produce industrially important substances. In India, *idli* batter fermentation has been the focus of research studies since a long time. A large number of LAB have been identified to be a part of the microflora responsible for fermentation of *idli* batter and they include *Leuconostoc mesenteroides, Lactobacillus coryneformis, L.delbrueckii, L. fermentum, L. lactis, Streptococcus faecalis,* and *Pediococcus cerevisiae* (Yajurvedi 1980). Areas such as preparation methods, nutritive value and microbiology of *idli* have been extensively studied and well documented (Desikachar et al. 1960; Steinkraus et al. 1967; Venkatasubbaiah et al. 1984; Kanchana et al. 2008; Riat and Sadana 2009; Sridevi et al. 2010). However, the probiotic potential of these microbes and their ability to produce industrially important substances has been seldom explored. Thus, in the current study isolates of LAB from *idli* batter (traditional Indian fermented food) were checked for their probiotic potential. Other health benefits and technological suitability of the isolates has also been studied.

Materials and methods

Materials Black gram (*Phaseolus mungo* L.) and parboiled rice used for making *idli* batter were procured from local market. Bile salts (Bile acids, min 70%) was obtained from SD Fine Chemicals, Mumbai, India. Vitamin B_{12} standard was procured from Centron Research Laboratories, Mumbai, X-gal (5-bromo-4-chloro-3- indolyl-β-D-galactopyranoside) and IPTG (isopropyl- thio-β-D-galactopyranoside) was purchased from Bangalore Genei, India. ONPG was procured from Merck, Mumbai. *E. coli* NRRL 3008 was obtained from NRRL, Agricultural Research Service Culture Collection, Peoria, USA. *Bacillus cereus* MTCC 1272 and *Listeria monocytogenes* MTCC 1143 were purchased from MTCC, Chandigarh. *E. coli* NCIM 2068 for Vitamin B_{12} assay was procured from NCIM, Pune. MRS and nutrient agar medium was purchased from HiMedia Labs Pvt. Ltd, India. All other chemicals used were of analytical grade.

Strain isolation and identification

Idli batter preparation and isolation The procedure for preparation of *idli* batter was as followed by Iyer and Ananthanarayan (2008). For isolation of LAB from *idli* batter, 10 g of the batter was added to 90 ml saline (0.9% w/v NaCl) and blended thoroughly on a orbital shaker for 30 min. Appropriate serial dilutions of the blended mixture was plated onto MRS agar (HiMedia Labs Pvt. Ltd., India) and incubated at 37°C for 48 h. This procedure was repeated at regular time intervals during the course of fermentation. Well isolated translucent/opaque colonies, 2–3 mm in diameter having entire margins were picked up and suspended in MRS broth and incubated at 37°C for 48 h. The process was repeated until pure cultures were obtained. These isolated LAB's were maintained on MRS agar slants, by subculturing them periodically and stored in 40% glycerol at −20°C.

Identification of the idli batter isolates The isolates obtained were checked for their Gram nature, morphology, catalase and antimicrobial activity. Two isolates exhibiting maximum antimicrobial activity (labeled as IB-1 and IB-2) were further characterized by physiological and biochemical tests according to Bergey's Manual of Systematic Bacteriology (Mundt 1986). These included the study of fermentation type, agar plug test, triple sugar utilization test and citrate utilization test. Sugar utilization ability of the isolates was also studied. Finally, IB-1 and IB-2 were sequenced for 16S rRNA (National Centre for Cell Science, Pune) to confirm the results obtained from biochemical characterization. The sequences of the selected isolates were submitted to GenBank (NCBI).

Determination of antimicrobial activity Antimicrobial activity was assayed by an adaptation of the critical dilution assay method (Mayr-Harting et al. 1972). The 48 h culture grown in MRS medium was centrifuged for 20 min at 4°C, 8,000 rpm and the supernatant thus obtained was adjusted to pH 5.5 (maximum antimicrobial activity was observed at pH 5.5) and used to determine antimicrobial activity. *B. cereus* MTCC 1272, *E. coli* NRRL 3008 and *L. monocytogenes* MTCC 1143 were used as indicator organisms. Nutrient agar 2% (10 ml) was overlaid with nutrient agar 1% (5 ml) inoculated with overnight grown culture

suspensions of the indicator organisms. The plates were allowed to solidify and wells of 6 mm diameter were punched into them with a sterile cork borer. Cell free extract (100 μl) was poured in each of the wells and the plates were placed in the refrigerator at 4°C for 20 min to enhance diffusion of sample. The plates were then incubated at 37°C for 24 h and examined for zone of inhibition. The antimicrobial activity was determined by measuring the zone of clearance around the wells (well diameter + zone of inhibition). The activities of the cell free fractions were classified as no inhibition zone (−), inhibition zone of 6.5–7.5 mm (+), inhibition zone of 7.5–9.0 mm (++), and inhibition zone >9.0 mm (+++). Each assay was performed in triplicates.

Bile tolerance Isolates IB-1 and IB-2 were grown in MRS broth containing 2% (w/v) of bile salts mixture at 37°C for 24 and 48 h. The growth was checked using the pour plate technique (Seeley and VanDemark 1971) wherein 1 ml of culture of appropriate dilutions was overlaid with MRS agar. The plates were incubated at 37°C for 48 h and the cell count was compared with that of the control MRS agar plates (containing cultures grown in MRS medium without bile salts mixture). Bacterial growth was expressed as colony forming units per milliliter (CFU/ml) and the survival percentage (% ± sd) of strains to bile salts was calculated as given below. (Mourad and Nour-Eddine 2006)

$$\% \text{survival} = \log \text{CFU}\, N_1 / \log \text{CFU}\, N_0 \times 100$$

Where,

N_1 is viable count after exposure to bile salts
N_0 is viable count without exposure to bile salts

Tolerance to acidic pH values Isolates IB-1 and IB-2 were grown in MRS broth at 37°C for 48 h. The cultures were centrifuged at 8,000 rpm for 10 min at 4°C. The pellets were washed twice in sterile phosphate-buffered saline (PBS), pH 7 and resuspended (1:100) in PBS to achieve a cell density of 1×10^{12} cells/ml. This was employed for setting up the experimental control and studying survival of isolates at low pH (pH 1, 2 and 3 prepared in PBS). The suspensions were incubated at 37°C and samples were removed after every 1 h up to 4 h. Counts of surviving cells were determined by plating on MRS agar using the procedure followed in bile tolerance assay. Bacterial growth was expressed in CFU/ml and the survival percentage (% ± sd) of strains to different pH values was calculated.

Lactic acid and biomass production A time-course experiment to study the growth and lactic acid production profiles of IB-1 and IB-2 was carried out. The isolates were grown in 30 ml of basal MRS medium, pH 6.5 (in 100 ml flasks). Flasks were removed every 6 h up to 54 h. For biomass determination, 2 ml of the cells were centrifuged and washed twice with distilled water and kept for drying at 60°C until constant weight was obtained. The rest of the media was centrifuged and the broth obtained was used for measuring lactic acid content and pH. The lactic acid content was measured using a modified spectrophotometric method by Pryce (1969) and expressed in mM. The pH of the fermented broth was measured directly by using a digital Equip-Tronics ® pH meter (Mumbai, India).

Screening for vitamin B_{12} and β- galactosidase activity of IB-1 and IB-2 Vitamin B_{12} production was checked by plate method using *E. coli* NCIM 2068, a vitamin B12 auxotroph. The *E. coli* was grown for 18 h in Harrison medium containing (in g/l) glucose, 30; K_2HPO_4, 21; KH_2PO_4, 9; sodium citrate, 15; $(NH_4)_2SO_4$, 3; asparganine; 0.6, MgSO4; 0.3 (Harrison et al. 1951). The flasks containing molten Harrison medium was inoculated at around 45°C with the 18 h old culture (O.D. 0.04 at 540 nm; 2% v/v) and immediately poured into sterile petri plates. The medium was allowed to solidify and 4 wells were punched in each of the plates using a sterile cork borer. Fermented broth (100 μl) was poured into two of the wells and a standard, filter sterilized Vitamin B_{12} solution (2–10 μg/ml) was poured into the other two wells. The plates were incubated at 37°C for 24 h after which the zone of exhibition was measured around the wells and compared with standard vitamin B_{12} solution (Atta et al. 2008)

For screening of β–galactosidase activity, the isolates were grown on MRS agar plates containing 0.01% X-gal (5-bromo-4-chloro-3- indolyl-β-D-galactopyranoside) and 0.1 mM IPTG (isopropyl- thioβ-D-galactopyranoside) as an inducer and the plates were incubated at 37°C for 48 h. Appearance of blue coloured colonies was indicative of β–galactosidase production (Karasová et al. 2002). For quantification of β–Galactosidase, the isolates were grown in modified MRS medium (glucose was replaced with lactose) at 37 ºC, 48 h. The bacterial cells were harvested by centrifugation at 8,000 rpm, 4°C for 15 min and washed twice in distilled water. The cells thus obtained were suspended in 0.2 M, pH 7 phosphate buffer and lysed by ultra sonication (Branson 450 Sonifier, Danbury, Connecticut., USA) set at 80% duty cycle, output 3 for 5 min to get a crude enzyme solution. The β–galactosidase activity of the abovementioned solution was determined using the o-NitroPhenylGalacto-Pyranoside (ONPG) method (Hestrin et al. 1976).

All the experiments were performed in triplicates (*n*=3) and the difference (standard deviation) in the readings was less than or equal to ±5%.

Results and discussion

Strain isolation and identification A total of 15 LABs were isolated at different time intervals during the course of fermentation of *idli* batter. Four bacterial isolates were obtained from black gram after soaking it for 4 h at 37°C. Six bacterial isolates were obtained from *idli* batter after 4 h of fermentation, and five bacterial isolates were obtained from batter after 16 h of fermentation. All the isolates were Gram positive in nature and catalase negative. Some of the cultures were bacilli (short rods), the others were cocci and one was a coccobacilli. Based on the Gram nature, morphology and catalase test, the cultures were observed to belong to the LAB family. One of the important features of a probiotic culture is its ability to kill pathogens which infect the gastrointestinal system. The isolates were also checked for their antimicrobial activity against *B. cereus* MTCC 1272*, L. monocytogenes* MTCC 1143 and *E. coli* NRRL 3008 which are commonly found food borne pathogens that infect the GI tract. The results showed that two of the fifteen isolates could inhibit the indicator organisms, however, at different inhibition levels. All these results have been compiled in Table 1. The isolate IB-2 showed antibacterial activity against all the three indicator organisms, whereas IB-1 showed activity against *B. cereus* MTCC 1272 and *E. coli* NRRL 3008. Several researchers have observed many of the LAB's to be capable of producing antimicrobial substances that are active against pathogenic bacteria (Topisirovic et al. 2006). The differences in inhibition potential among the selected isolates could be due to different intrinsic factors induced by food origins (Klayraung et al. 2008).

Biochemical characterization and sugar utilization studies of IB-1 and IB-2 was carried out (Tables 2 and 3). IB-1 was found to be heterofermentative while IB-2 was homofermentative. Both the isolates seemed to follow mixed type fermentation and could not utilize citrate as carbon source. The sugar utilization pattern showed that the isolates could belong to *L. plantarum* (bacilli) and *L. lactis* (cocci) (Mundt 1986). The two isolates were subjected to 16S rRNA sequencing and the cultures were found to have 99% sequence similarity to that of *L. plantarum* (IB-1) and *L. lactis* (IB-2). The sequences were uploaded in NCBI and have been given accession number GU797249 and GU797248 for IB-1 and IB- 2, respectively.

In vitro evaluation as a probiotic

Bile tolerance of IB-1 and IB-2 One of the important criteria to be fulfilled for a LAB to be used as a probiotic is its ability to resist the effect of bile salts in the gastrointestinal tract (Lee and Salminen 1995). However, there are no reports on the exact concentration to which a selected strain should be tolerant. The physiological concentration of bile salts in the small intestine is anywhere between 0.2 and 2.0% (Gunn 2000). Therefore the isolates were treated with 2% bile as it is the highest concentration obtained in animal and human intestine during digestion process (Gotcheva et al. 2002). Both the isolates showed good resistance (IB-1: 58.11% and IB-2: 60.84%) to 2% bile salt even after exposure for 48 h. Resistance to bile is related to bile salt hydrolase (BSH), an enzyme which helps in hydrolysing conjugated bile, thus reducing its toxic effect (Du Toit et al.

Table 1 General characteristics and antimicrobial activity of the isolated strains

Isolate no.	Cell morphology	Antimicrobial activity[a]		
		E. coli	*B. cereus*	*L.monocytogenes*
1	Bacilli	+	+	−
2	Bacilli	+	+	−
3	Bacilli	+	+	−
4	Bacilli	+	+	−
5	Bacilli	+	++	−
6	Cocci	+	+	+
7	Bacilli	+	+	−
8	Cocci	++	+	−
9	Bacilli	+	+	−
10	Cocci	+	+	−
11 (IB-1)	Bacilli	+++	+++	−
12 (IB-2)	Cocci	+++	+++	++
13	Coccobacilli	+	+	−
14	Cocci	−	−	−
15	Bacilli	+	+	−

[a] Antimicrobial activity: (−) no inhibition zone, (+) 6.5–7.5 mm inhibition zone, (++) 7.5-9 mm inhibition zone, (+++) >9 mm inhibition zone. 2 selected strains were labelled as IB-1 & IB-2. *B. cereus* MTCC 1272, *E. coli* NRRL 3008 and *L. monocytogens* MTCC 1143. (n=3)

Table 2 Biochemical characterization of the isolates IB-1 and IB-2

Tests	Medium used	Results	
		IB-1	IB-2
Fermentation type	Glucose phosphate broth	MR positive (Mixed acid fermentation)	
Agar plug test	Homo –Hetero fermentative medium	Gas formation (Heterofermentative)	No gas formation (homofermentative)
Triple sugar utilization	TSI agar slants	Yellow coloured slant and butt (Utilizes all sugars without H_2S & gas. No utilization of N source after exhaustion of C source)	
Citrate utilization	Simmon's citrate utilization agar tests	No change in colour (does not use citrate as carbon source)	

(*n*=3)

1998). This differs significantly among the LAB species and their strains. Similar results were also reported by Mourad and Nour-Eddine (2006) who found one of their isolated strains *L. plantarum* OL 16 to show 65% survival rate on exposure to 2% bile salt.

Acid tolerance of IB-1 and IB-2 A probiotic strain should survive transit through the stomach where pH can be as low as 1.5 to 2. Hence, tolerance to extremely acidic conditions is another important feature of a probiotic strain (Dunne et al. 2001, Guo et al. 2009). Figure 1 summarizes the results of acid tolerance (survival percentage) of IB-1 and IB-2 at pH 1.0, 2.0 and 3.0 studied for a period of 1, 2, 3 and 4 h. Throughout the study, it was seen that IB-1 (*L. plantarum*) showed better survival as compared to IB-2 (*L. lactis*). It was observed that at pH 3.0, IB-1 and IB-2 showed survival of 88.13% and 89.85%, respectively, even after 4 h of incubation. However, it was noted that the percentage of survivors of both the isolates decreased with a decrease in pH. The isolate IB-1 (*L. plantarum*) showed higher survival up to 3 h (64.41%, 44.11%, and 24.96% at 1, 2 and 3 h of exposure, respectively) as compared to IB-2 (*L. lactis*) which survived only up to 2 h (69.98% and 54.41% at 1 and 2 h of exposure, respectively) at pH 2.0. At pH 1.0, IB-1 showed 53.17% survival after 1 h and 27.51% survival after 2 h; IB-2 showed 48.69% survival after 1 h. Mourad and Nour-Eddine (2006) had also reported their *L. plantarum* strains isolated from olives to show better survival rates at acidic pH as compared to other strains. Many researchers have attributed the acid tolerant nature of LAB to induction of H^+-ATPase activity (Matsumoto et al. 2004, Ventura et al. 2004). Therefore, the difference in the acid tolerance of the isolates IB-1 and IB-2 might be related to the difference in H^+-ATPase activity of the two strains. Both the isolates survived at upto 4 h at pH 3.0.

Evaluation of the potential of IB-1 and IB-2 as an industrial strain

Biomass and lactic acid production by IB-1 and IB-2 Figure 2 a & b depicts the biomass production in terms of dry cell weight (DCW) and lactic acid production (mM) profile of isolates IB-1 and IB-2. The time-course study was conducted to compare the growth and lactic acid producing capacity of lactobacilli and lactococci strain. The growth of both the isolates increased steadily upto 54 h at the end of which the DCW was 0.65 and 0.58 g/l for IB-1 and IB-2, respectively. It was observed that during the first 6 h, the lactococci strain IB-2 grew faster than the lactobacilli strain (IB-1). However, between 6 and 54 h the lactobacilli strain (IB-1) grew better than the lactococci strain (IB-2). A similar trend was observed in case of lactic acid production. The lactic acid content at the end of 54 h was 181.93 and 153.44 mM for IB-1 and IB-2 respectively.

Table 3 Carbon source utilization of the isolates *L .plantarum* (IB-1) and *L .lactis* (IB-2)

Strain	Carbon source														Identified species
	Mal	Xyl	Cel	Ara	Tre	Sal	Sor	Suc	Raf	Rha	Mant	Mann	Meli	Gal	
IB-1	+	V	V	V	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
IB-2	+	−	V	+	+	+	−	+	−	−	−	+	−	V	*Lactococcus lactis*

Mal maltose; *Xyl* xylose; *Cel* cellibiose; *Ara* arabinose; *Tre* trehalose; *Sal* salicilin; *Sor* sorbitol; *Suc* sucrose; *Raf* raffinose; *Rha* rhamnose; *Mant* mannitol; *Mann* mannose; *Meli* mellibiose; *Gal* galactose.

V Variable

(*n*=3)

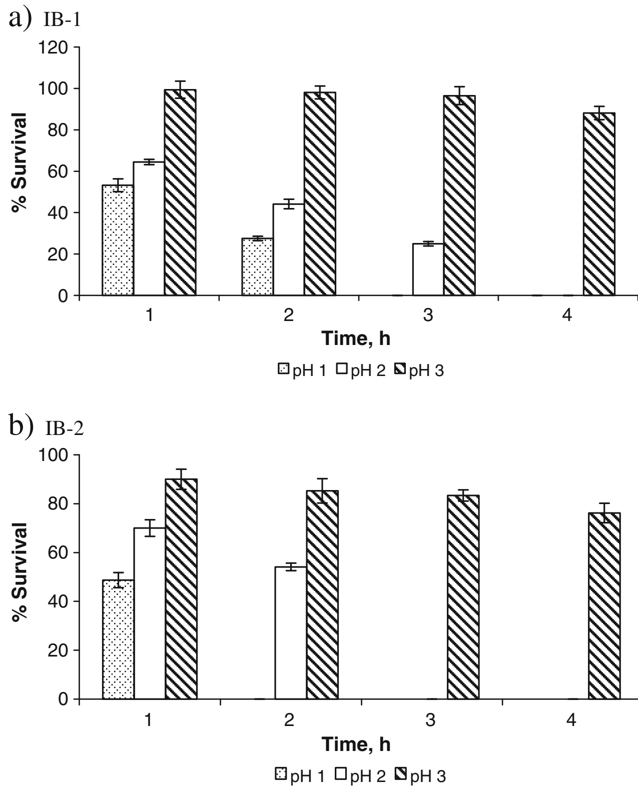

Fig. 1 Tolerance of **a** IB-1 (*L. plantarum*) and **b** IB-2 (*L. lactis*) to acidic pH (n=3)

It was noticed that lactic acid production of both the isolates were parallel with that of biomass production. These results show that lactococci (IB-2) was less tolerant to lactic acid than lactobacilli (IB-1) and therefore there was lesser biomass produced in case of IB-2 strain. This confirmed the effect of lactic acid on biomass production. At industrial level, this problem could be solved by using a strategy wherein pH can be adjusted so as to help higher accumulation of lactic acid and biomass. A similar trend, wherein production of lactic acid limited growth of LAB was also observed by Adnan and Tan (2007). However in their study the amount of lactic acid produced was less and amount of biomass produced was higher, contrary to the results of our study.

Vitamin B_{12} and β-galactosidase production by IB-1 and IB-2 Vitamin B_{12} is an essential cofactor in amino acid, carbohydrate, fatty acid and nucleic acid metabolism, not produced by our body naturally but is required in good amounts for maintaining good health. It is predominantly of microbial origin, and is found in foods such as milk, meat and egg (Quesada-Chanto et al. 1994). In addition to dietary sources, intestinal microflora contributes to vitamin B_{12} in humans. A zone of exhibition of *E. coli* NCIM 2068 around the wells containing cell- free extracts of IB-1 and IB-2 broth indicated production of vitamin B_{12} by the isolates. A zone of growth was also observed surrounding the wells containing standard vitamin B_{12}, as was expected. On comparing the zone of exhibitions of the standard and the sample it was seen that the broth contained approximately 8 μg/ml of Vitamin B_{12}. Madhu et al. 2010 isolated an intracellular Vitamin B_{12} producing *L. plantarum* strain from *Kanjika*; however the selected isolates used in our study produced vitamin B_{12} extracellularly. No reports of an *L. lactis* strain producing Vitamin B_{12} have been reported so far. The reason behind the isolates producing Vitamin B_{12} maybe due to the presence of B_{12} biosynthetic gene cluster which encodes the enzymes required for the synthesis of this important vitamin (Santos et al. 2008). This could be probed further for industrial production of vitamin B_{12}.

β-galactosidase, is an enzyme widely used in dairy industry. This enzyme is beneficial in preparation of lactose-free milk and biosynthesis of galactooligosaccharides that are important biomolecules from industrial and health point of view. In the current study it was observed that both the isolates IB-1 and IB-2 formed characteristic blue colored colonies due to hydrolysis of X-Gal. The ability of the isolates to hydrolyse X-gal (chromogenic substrate) in the medium qualitatively indicates the presence of β-galactosidase. Further these isolates when grown

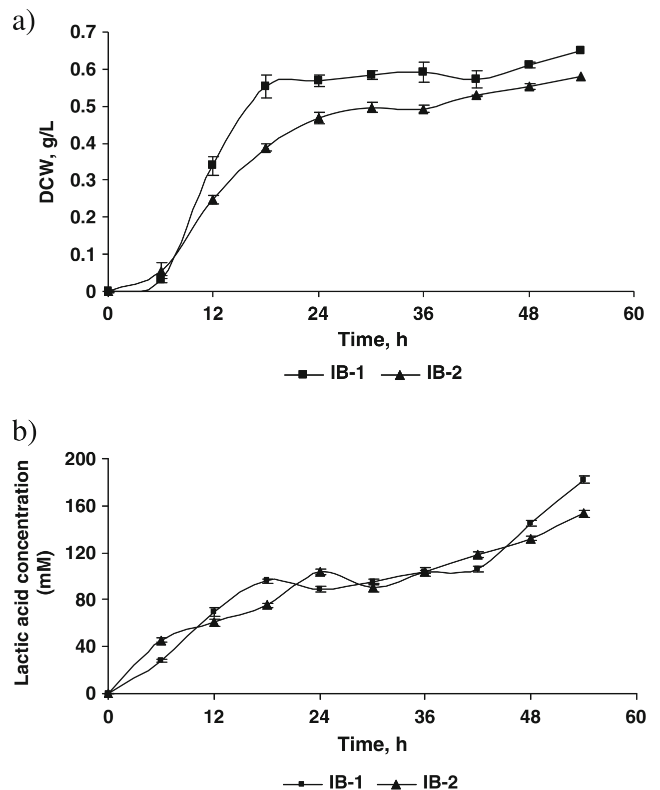

Fig. 2 **a** Biomass production (DCW/l) profile of the two isolates IB-1 and IB-2. **b** Lactic acid production (mM) profile of the two isolates IB-1 and IB-2 (n=3)

in liquid medium produced 0.5 U/ml of the enzyme. Researchers have reported presence of β-galactosidase activity in LAB (Aysun and Candan 2003). Evidences support the claim of lactose-intolerant individuals being able to tolerate fermented lactose rich dairy products better; these beneficial effects were attributed to microbial β-galactosidases (Vasiljevic and Shah 2008). These strains could be further explored for production of the enzyme.

Conclusion

L. plantarum (IB-1) and *L. lactis* (IB-2) strains isolated from fermented *idli* batter could tolerate high bile salt concentration and low pH. Based on these in vitro tests, there is high possibility that the isolates would be able to reach the intestinal tract in good numbers. Both the isolates were good lactic acid producers and also showed antibacterial activity against pathogenic microorganisms. The ability of the isolates to produce vitamin B_{12} and β -galctosidase was an added advantage as both of these are essential in improving digestion and metabolism. This could be considered a positive trait for microorganisms which are used as starter cultures and in manufacturing of probiotic and novel functional foods. However, the isolated strains need to be further investigated using in vivo experiments to establish their potential health benefits.

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