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Research Article

Comparison and evaluation of functional properties of potential probiotic cultures by *in-vitro* tests

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Abstract

Probiotic culture *Lactobacillus helveticus* MTCC 5463 (V3) and *Lacticaseibacillus rhamnosus* MTCC 5462 (I4) were used for this study for comparison and evaluation of functional properties. Cultures were inoculated @ 2% for conducting each study. V3 culture reduced pH from 6.36±0.02 to 4.8±0.09 in 24th h of observation. I4 reduced pH from 6.3±0.05 to 5.01±0.06 in 24th h. I4 was more tolerant to bile salt than V3. It showed mean Abs_{620} - 0.435 compared to V3 Abs_{620}- 0.395 at 0.3% bile concentration. In case of phenol tolerance, V3 was tolerant to phenol concentration of 0.4%, whereas I4 showed tolerance up to 0.4% phenol. I4 showed more tolerance to pancreatin than V3. Both were tolerant to 0.3% pancreatin, but I4 showed higher Abs_{600} (0.433) than V3 (0.301). The cell surface hydrophobicity values for V3 ranged from 47.31 to 78.06% and for I4 it remained 33.29 to 67.88% to n- hexadecane, n- hexane, xylene and chloroform. In case of auto-aggregation values increased over incubation period and ranged from 21.08 - 51.22% and 25.47- 43.14% for V3 and I4 respectively. V3 showed good auto aggregation property than I4. V3 (10.92 log cfu/mL) showed a significantly higher viability than I4 (10.59 log cfu/mL). There was a significant increase in cell viability for both of the cultures up to 24 hrs. According to statistical analysis V3 culture showed comparatively better results for conducted *in-vitro* tests than I4.

Keywords: Auto-aggregation, Bile tolerance, Cell surface hydrophobicity, Phenol tolerance, Probiotics

Highlights

- *Lactobacillus helveticus* MTCC 5463 (V3) was more tolerant to phenol concentration than *Lacticaseibacillus rhamnosus* MTCC 5462 (I4) in 0.4% phenol.
- V3 culture showed more tolerance to pancreatin than I4. Both analysed cultures V3 and I4 were tolerant to 0.3% pancreatin.
- V3 showed good auto aggregation property than I4.
- V3 (10.92 log cfu/mL) showed a significantly higher cell viability than I4 (10.59 log cfu/mL).
- There was a significant increase in cell viability for both of the cultures up to 24 hrs, indicating V3 is better culture than I4 and can be used to formulate probiotic products.

INTRODUCTION

The use of foods containing probiotics is constantly increasing because these foods are said to improve human health (Crittenden and Playne, 2008; Stanton *et al*., 2001). Functional probiotic foods have been manufacturing all around the continents and studies on human candidates have shown that they have favourable impacts on health (Raghuveer and Tandon, 2009). Functional food is a staple diet which gives added benefits in addition to its conventional nutritional value. Its beneficial effects are mainly attributed by the occurrence of physiologically active components; those might have potential to reduce the risk of chronic diseases (Helmy, 2012).

Restoring microbial balance is the basis for utilizing probiotics. The adult digestive system is a home to more than 500 distinct bacterial species. While certain bacteria are thought to be useful to the human being as probiotics and others are pathogenic and spoilage-causing organisms. Though, constant use of antibiotics, immune-suppressive drugs and irradiation can increase the population of harmful bacteria and upset this equilibrium, causing an optimal balance of gut flora to be disrupted. This microbial equilibrium in the

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gastrointestinal system may be restored with probiotic lactic acid bacteria (LAB) and yeasts (Doron and Gorbach, 2006). Probiotics need to have specific qualities in order to be effective *i.e.* it should be able to survive their transit through the digestive tract, must adhere to the intestinal epithelial lines, colonize and multiply there, and keep the balance of the gut flora as well as it must be safe for human consumption (Goldin, 1998). Current study focuses on screening of probiotic cultures based on some functional properties that can be helpful in selecting most suited culture according to its use.

MATERIALS AND METHODS

LAB cultures and their maintenance: *Lactobacillus helveticus* MTCC 5463 (V3), *Lacticaseibacillus rhamnosus* MTCC 5462 (I4) were obtained from the culture collection of Dairy Microbiology Department, SMC College of Dairy Science, Kamdhenu University, Anand. The LAB cultures were propagated in sterilized reconstituted skim milk (12% TS) and stored at 5 ± 2°C. Regular transfer was given to each culture in an interval of 20 days during the course of the study.

pH change: This test was carried as described by Bodyfelt *et al*. (1988) to get a better understanding of pH lowering capacity of the selected probiotic culture during a period of 24 h. Change in pH was measured at the interval of 6 hrs (0, 6, 12, 18 and 24th h) at 37 °C.

Bile salt tolerance: Bile salts tolerance of the cultures was determined by the spectrophotometric method as described by Jawan *et al*. (2019). Growth of the cultures was measured on the basis of optical density (OD) at 620 nm in every 6 hrs of interval of 24 h of observation.

Phenol tolerance: Phenol tolerance of the cultures was determined by direct observation method as described by Patidar, (1995). Growth of the cultures was measured on the basis of curd formation and body of the curd in interval of every 6 h up to 24 h of observation.

Pancreatin tolerance: Pancreatin tolerance of the cultures was determined by the spectrophotometric method as described by Khagwal *et al*. (2014). Growth of the cultures was measured on the basis of optical density (OD) at 600 nm in every 6 h of interval of 24 h of observation.

Cell surface hydrophobicity: The procedure of Kodaikkal (2008) has been used with slight modification. In this study, we have used four hydrocarbons (Xylene, N-hexadecane, N-hexane, and Chloroform) to get a better confirmation of the hydrophobicity of the respective cultures. The cell concentration was adjusted with saline to OD_{600} to 0.5 ± 0.070, to get initial reading (A_0).

Auto-aggregation property: Method for preparing cell suspension is similar to that of cell surface hydrophobicity. The analysis was carried out as method followed by Kodaikkal (2008) with slight modification.

Cell viability: Cell viability or Lactobacillus counts of respective cultures were determined as per the method described by IS 1479-3, 1977. Typical colonies were calculated and the counts were expressed as log cfu/mL.

Statistical analysis

Statistical analysis was carried out using statistical design CRD and FCRD (Steel and Torrie, 1980). All the experiments were conducted in required numbers of replications and the results are expressed as mean± standard deviation (SD).

RESULTS

pH change: Freshly grown cultures were inoculated

Table 1. Change in pH of MRS broth by LAB strains

Treatment (T)	Incubation period (P, h)					Treatment-mean (T)
	0	6	12	18	24	
C	6.51±0.00	6.50±0.01	6.49±0.02	6.46±0.05	6.46±0.04	6.48^{b}
V3	6.36±0.02	5.88±0.12	4.86±0.03	4.79±0.02	4.80±0.09	5.34^{a}
I4	6.3±0.05	5.55±0.12	4.97±0.02	4.94±0.02	5.01±0.06	5.35^{a}
Period Mean (P)	6.39^{C}	5.98^{B}	5.44^{A}	5.4^{A}	5.42^{A}	
Source		**SEm±**		**CD (0.05)**		**CV (%)**
T		0.023		0.070		1.642
P		0.030		0.090		
T x P		0.051		0.156		

Each observation is mean ± standard deviation of three replicates

Mean with different superscripts (a, b) within column and superscripts (A, B, C) within row differ significantly ($p<0.05$)

@ 2% (v/v) and kept for incubation at 37ºC for 24 hrs in MRS broth and pH of the media was checked at every 6 hrs of interval during 24 hrs of period. MRS broth without addition of culture was kept as control sample.

From Table 1, it was observed that reduction in pH of the MRS growth media was by LAB isolates were significant ($p<0.05$) till 6th h of observation and further pH was reduced till 24th h of observation but it was non-significant. In the 6th hrs, in case of V3, pH was reduced from 6.36±0.02 to 5.88±0.12 and further lowered down to 4.8±0.09 in 24th hrs of observation. In the scenario of I4, the reduction pattern was similar to that of V3, as pH was reduced from 6.3±0.05 to 5.55±0.12 in the first 6 hrs ($p<0.05$) and was further reduced to 5.01±0.06 in 24th hrs of observation.

Bile tolerance: Bile tolerance was carried out in different bile concentrations (0.1, 0.2, 0.3, 0.4 and 0.5%) at 37ºC for a period of 24 hrs.

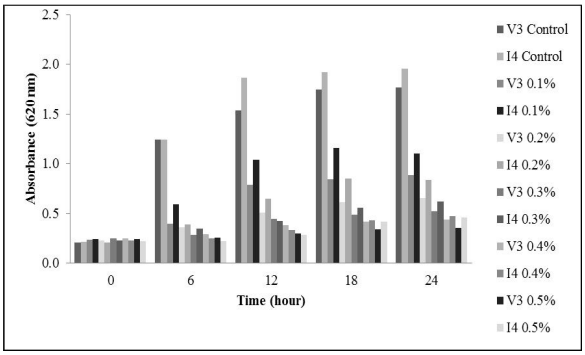

Fig. 1. Comparison of bile tolerance on the growth of V3 and I4

Fig. 1 shows bile salt tolerance of *L. helveticus* MTCC 5463 and *Lactobacillus rhamnosus* MTCC 5462. According to it, *L. helveticus* MTCC 5463 was significantly tolerant ($p<0.05$) to bile salt concentration of 0.3% and there was no significant difference in growth when incubated with 0.3% and 0.4% of bile salt. Eventually, it did not exhibit an increase growth in 0.5% bile concentration.

Periodically there was a significant increase in growth up to 18th h while the growth in 24th h of incubation was not significantly different from 18th h sample. While in case of control sample, in 6th h the absorbance was as 1.245±0.05, which further increased to 1.744±0.05 and 1.769±0.05 in the sample incubated at 18th and 24th hrs of incubation.

As indicated, the growth of *Lactobacillus rhamnosus* MTCC 5462 against different bile concentration is shown. In case of I4, it showed good growth characteristics up to 0.5% bile concentration. Growth was increasing in terms of absorbance up to 0.4% bile and it was similar in 0.5% bile.

Up to 18 hrs of interval, it was growing significantly and the values in 18th and 24th h were found to be non-significant. In 6th h, 1.241±0.06 absorbance was observed in case of control sample which during same time interval was observed to 0.221±0.04 in sample containing 0.5% bile salt. When compared 24th h absorbance of the control sample with other added bile alts, it was 1.952±0.07 in case of control while growth of culture decreased to 0.457±0.05 in case of 0.5% bile salt.

Phenol tolerance: Phenol tolerance was carried out in different phenol concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 %) at 37ºC for a period of 24 hrs. Growth of the cultures was measured on the basis of curd formation in previously mentioned time intervals.

It was observed that with increase in addition rate of phenol there was a delay in curd formation. In case of V3, firm curd was formed in 12th h at 0.1 and 0.2 % phenol concentration. At 0.3% and 0.4% concentration firm curd was formed at 18th h and 24th h respectively. In sample containing 0.5% phenol concentration, very weak curd formation was observed even after 24 hrs.

In case of I4 culture, firm curd was formed at 18th h of observation up to 0.3% of phenol concentration and very weak curd was formed in 0.4 and 0.5% of phenol. According to time of curd formation V3 showed good results in contrast to I4.

Pancreatin tolerance: Pancreatin tolerance was carried out in different pancreatin concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 %) at 37ºC for a period of 24 hrs. Effect of pancreatin concentrations on growth of V3 and I4 is given in Fig. 2.

Fig. 2 shows that treatment mean of *L. helveticus* (V3) was significantly tolerant ($p<0.05$) to pancreatin

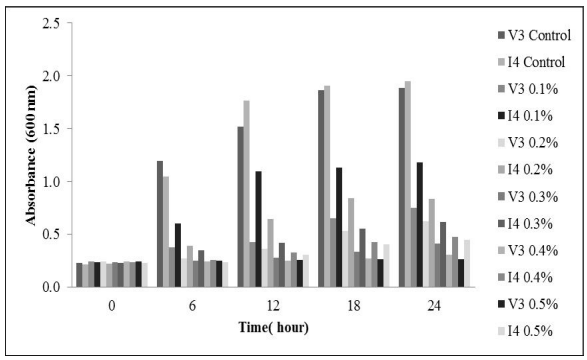

Fig. 2. Comparison of pancreatin tolerance on the growth of V3 and I4

concentration of 0.3%, showing good growth and in 0.4 % and 0.5 % of pancreatin concentration, growth was non-significant. There was a minimal growth of V3 in 0.5 % pancreatin. Periodically there was a significant increase in growth up to 24th h. A gradual increase in growth was observed.

Culture I4 in accordance with treatment mean, was tolerant ($p<0.05$) to 0.3% pancreatin concentration while growth in 0.4% and 0.5% of pancreatin concentration was non-significant. When compared period mean values, there was a significant increase in growth up to 24th h and a gradual increase was observed.

Cell Surface Hydrophobicity (CSH): For checking CSH four hydrocarbons were used after 1 h of incubation at 37ºC. The cell surface hydrophobicity of selected lactobacilli cultures presented as percentage hydrophobicity is shown in Table 2. The CSH % values for V3 ranged from 47.31 to 78.06 % and for I4 it remained 33.29 to 67.88 % to n- hexadecane, n- hexane, xylene and chloroform respectively.

By comparing with the % CSH value with four hydrocarbons only in case of xylene I4 showed higher value (59.10±0.06) than V3 (47.31±0.08). In case of other three hydrocarbons, V3 was found to have significantly higher hydrophobicity than I4.

Auto- aggregation assay: Bacterial cell suspension was mixed vigorously for one minute and auto-aggregation was determined during a period of 24 hrs at every 6 h of interval at 37ºC.

The results of auto-aggregation study are as shown in the Table 3. In the results recorded by performing the auto-aggregation of LAB isolates during a period up to 24 h, the rate of aggregation was found to be increasing with time.

Both the isolates showed significant difference ($p<0.05$) in auto-aggregation values in terms of percentile on 6th, 18th and 24th h of interval. On 12th, there was no significant difference found. Individually the values increased over incubation period and ranged from 21.08 - 51.22% and 25.47- 43.14% for V3 and I4 respectively. V3 showed good auto aggregation property than I4.

Table 2. Cell Surface Hydrophobicity (%) of V3 and I4

Cultures	n- hexadecane	n- hexane	Xylene	Chloroform
V3	55.86^{b}±0.043	78.06^{b}±0.02	47.31^{a}±0.08	51.35^{b}±0.03
I4	33.29^{a}±0.03	67.88^{a}±0.05	59.10^{b}±0.06	43.32^{a}±0.02
SEm±	2.13	2.12	2.44	1.52
CD (0.05)	8.59	8.54	9.82	6.11
CV %	8.27	5.05	7.93	5.55

Each observation is mean ± standard deviation of four replicates Values with different superscripts within a column differ significantly ($p<0.05$)

Table 3. Comparison of auto aggregation (%) property of V3 and I4

Treatment	Incubation period (h)				
	0	6	12	18	24
V3	0±0.00	21.08^{a} ±0.02	33.40±0.01	47.85^{a} ±0.01	51.22^{a} ±0.01
I4	0±0.00	25.47^{b} ±0.01	32.62±0.02	37.79^{b} ±0.03	43.14^{b}±0.04
SEm±	0	0.768	0.742	1.108	1.82
CD (0.05)	0	3.095	NS	4.469	7.339
CV %	0	5.712	3.896	4.481	6.682

Each observation is mean ± standard deviation of three replicates Values with different superscripts within a column differ significantly ($p<0.05$)

Table 4. Comparative study of cell viability of V3 and I4

Treatment (T)	Probiotic count (log cfu/mL) at different incubation periods (P, h)					Treatmentmean (T)
	0	6	12	18	24	
V3	8.22±0.11	9.41±0.08	11.27±0.17	12.24±0.19	13.48±0.08	10.92^{a}
I4	8.38±0.15	9.86±0.53	10.56±0.38	11.63±0.37	12.54±0.05	10.59^{b}
Period mean (P)	8.3^{A}	9.63^{B}	10.92^{C}	11.93^{D}	13.01^{E}	
Source		**SEm±**		**CD (0.05)**		
T		0.068		0.167		
P		0.107		0.264		
T x P		0.151		0.374		
CV (%)			2.005			

Each observation is mean ± standard deviation of three replicates

Mean with different superscripts (a, b) within a column and superscripts (A, B, C, D, E) within a row differ significantly ($p<0.05$)

Cell viability: Lactobacillus counts were done in every 6 h interval during a period of 24 h by pour plate method. Typical colonies were calculated as per the standard protocol and the counts were expressed as log cfu/mL.

As shown in Table 4 while comparing the treatment mean (T) a significant difference was observed ($p<0.05$) between V3 and I4 cultures. V3 isolate showed significantly higher viability than I4 isolate. Mean viability of V3 was 10.92 log cfu/mL while I4 had a viability of 10.59 log cfu/mL. Comparing period means a significant increase in cell viability for both of the cultures were observed with time interval increased. For V3 isolate it increased from 8.22±0.11 to 13.48±0.08 log cfu/mL and in case of I4 isolate it increased from 8.38±0.15 to 12.54±0.05 log cfu/mL.

DISCUSSION

pH change: As reported in literature strains of *Lactobacillus helveticus and Lacticaseibacillus rhamnosus*are good in acid production and significant amount of pH drops reported in suitable growth medium. Elfahri *et al*. (2016) evaluated effects of some selected *Lactobacillus helveticus* strains on anti-colon cancer and antioxidant activities. The bovine skim milk was fermented with four different strains of *L. helveticus* and pH was measured during different time intervals during 24 hrs. Among all studied strains, *L. helveticus* L1188 reduced pH of milk to 4.92 ± 0.18 that was further reduced to 3.48 ± 0.14 after 24 hrs. In a comparative growth behaviour of lactic acid bacteria, Hati *et al*. (2018) showed decline of pH in skim milk and soy milk by some LAB strains. Within those selected strains, *Lactobacillus bulgaricus* NCDC 09 reduced pH of skim milk to 2.76 after 24 hrs and *Streptococcus thermophilus* MD2 reduced pH of soy milk to 4.43 after 24 hrs. Obtained values were the maximum reduction of pH in skim milk and soy milk respectively.

In the present investigation also, V3 reduced pH from 6.36±0.02 to 4.86±0.03 ($p<0.05$) and further lowered down to 4.8±0.09 in 12th and 24th hrs of observation respectively. I4 reduced pH from 6.3±0.05 to 4.97±0.02 in the first 12 hrs ($p<0.05$) and was further reduced to 5.01±0.06 in 24th h. Treatment wise reduction of media pH was non-significant.

Bile salt tolerance: It is expected for a potential probiotic strain to fulfil basic survival skill in gut. This can be ascertaining *via* some *in vitro* tests – bile salt hydrolase activity, resistance to gastric pH, bile acid resistance, antimicrobial activity against potential pathogens and ability to diminish pathogen adhesion to inner gut lining. These assays are designed to replicate the hostile gut environment in an *in vitro* setting. Before clinical studies are carried out on human subjects, the cultures that were determined to be probiotics based on these assays should undergo preclinical validation in the relevant animal models. In present study also, I4 and V3 showed good resistance to bile salts up to 0.3 % addition up to 24 hrs of incubation period. I4 showed better growth than V3 (Fig. 1).

Gardiner *et al*. (2002) examined *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 for bile tolerance for 24 hrs at concentrations of 0, 0.15, 0.3, 0.5, 0.8, and 1.0% by). For GR-1 and RC-14, viability was not reduced up to bile concentrations of 0.5 and 0.3% (w/v), respectively. A 0.3% bile concentration is regarded as physiologically significant. Menconi *et al*. (2014) tested the bile tolerance of a few LAB isolates at bile concentrations of 0%, 0.4%, 0.5%, and 0.6%. When LAB 18 and LAB 48 were inoculated at bile salt concentrations of 0.4%, 0.5%, and 0.6% at 2, 4 and 24 hrs of incubation, they were able to grow.

The probiotic properties of *Lactobacillus helveticus* and *Lactobacillus plantarum* isolates that produce bacteriocin were evaluated by Hassan *et al*. (2020) from traditional Pakistani yoghurt. The findings revealed that the presence of bile salts up to 0.3% caused the isolates to develop at their fastest rate. The isolates, however, were less resistant to 0.6% and 1% concentrations. Maximum tolerance was seen in *Lactobacillus helveticus* isolates at 0.1%, and tolerance ranged from 0.3% to 0.1%.

Phenol tolerance: When certain amino acids are deaminated, microorganisms in the GIT release phenol, a harmful toxin. Therefore, in order to thrive in gut environment, prospective probiotic strains should be able to withstand phenolic condition. LAB are reported to have varying rate of phenol tolerance (Abdel *et al*., 2023).

It was observed that with increase in addition rate of phenol, there was a delay in curd formation. V3 was tolerant to phenol concentration of 0.4%, whereas I4 formed very weak curd in 0.4% phenol. According to time of curd formation, V3 showed good results in contrast to I4.

Some workers tested phenol tolerance in skim milk medium and reported similar type of results and confirmed phenol tolerance of LAB cultures. Khedkar (1988) conducted phenol tolerance of LBKV3 and LBKI4 isolates in skim milk medium. Both grew in

0.4 % phenol and LBKV3 was resistant to 0.5 % phenol whereas LBKI4 was tolerant up to 0.5 % phenol. Patidar (1995) tested four LAB isolates for phenol tolerance. Those showed tolerance up to 0.6% phenol and showed good growth till 0.2% of phenol concentration.

Sixty-seven LAB strains were tested to phenol tolerance by Bajaj *et al*. (2021) at three different concentrations (0.2, 0.4, and 0.6 %, w/v) for a 24-hour period. Only 18 of the 67 LAB isolates demonstrated growth (absorbance of 0.1 or above) at 0.4% of phenol. The growth of every isolate decreased as the phenol content increased (to 0.6%).

Pancreatin tolerance: In present study, I4 showed more tolerance to pancreatin than V3. Both were tolerant to 0.3% pancreatin, but I4 showed higher Abs_{600} (0.433) ($p<0.05$) than V3 (0.301).

Koll *et al*. (2010) examined pancreatin tolerance (0.5%, w/v) of 76 isolates. Out of those, 74 isolates shown good resistance to pancreatin and achieved a high score. Khagwal *et al*. (2014) investigated how pancreatin affected the isolates' ability to survive. After taking pancreatin concentration 0.5% (w/v), the trial was carried out for 48 hrs. All of the isolates were able to survive pancreatin, and some of them grew exceptionally well, while others grew slowly for up to 24 hrs. After 24 hrs of incubation, strain NKY1h1 had average growth, but after 48 hrs, the growth considerably enhanced.

Cell surface hydrophobicity: The Cell Surface Hydrophobicity (CSH) % values indicated that V3 was found to have significantly higher hydrophobicity than I4.

Several researchers had investigated the hydrophobicity of LAB using various hydrocarbons. Kodaikkal (2008) observed a significant range in hydrophobicity in a study using xylene. *L. acidophilus* strain 22A displayed the highest hydrophobicity (63.64%) followed by I4 (53.83%), LB1 (45.65%) and V3 (42.14%). Kathiriya (2014) reported the hydrophobicity percentage of *L. rhamnosus* NS6 to be 36.90% using n-hexadecane as hydrocarbon.

Rokana *et al*. (2018) evaluated cell surface hydrophobicity of eleven *Lactobacillus* isolates from human milk. Five hydrocarbons, including toluene, xylene, chloroform, n-hexane, and n-octane, were used to test hydrophobicity. Among eleven tested *lactobacilli*, only three strains (HM1, HM9 and HM12) exhibited higher hydrophobicity in comparison to others with all the five hydrocarbons. Hydrophobicity of HM1 isolate showed a value of 70·49 ± 5·64% with n-Hexane that was the highest value recorded.

Auto-aggregation property: Individually the values of Auto-aggregation increased over incubation period and ranged from 21.08 - 51.22% and 25.47- 43.14% for V3 and I4 respectively. V3 showed good auto aggregation property than I4.

The probiotic *L. acidophilus* M92's auto-aggregation property was investigated by Kos *et al*. in 2003. Over the course of 5 hrs, the aggregation value was measured, and it was discovered that the strain had a maximum aggregation of 74% and was entirely pH- and strain-dependent.

Using absorbance values at 600 nm, Collado *et al*. (2005) examined the auto-aggregation of many probiotic and pathogenic strains at 20ºC and 37ºC. This demonstrated that adhesion values gradually increased over the course of 24 hrs and aggregation values enhanced at a temperature of 37ºC. The probiotic bacteria demonstrated the highest rate of aggregation (73%), compared to the pathogenic strains examined.

Candida glabrata, which causes vulvovaginal candidiasis (VVC), Chew *et al*. (2015), investigated the antagonistic effects of the probiotic strains *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14. In this study, the auto aggregation assay was used to examine the various surface features of both strains. When compared to *Lactobacillus rhamnosus* GR-1 at 4 and 24 hrs (21.23±5.31% and 60.31±2.58% respectively), *Lactobacillus reuteri* RC-14 demonstrated a sturdier ability to form auto-aggregates and showed significantly additional auto-aggregation rates both at 4 and 24 h incubation *i.e*. 31.44±2.30% and 67.80±1.08%, respectively.

Cell viability: In case of cell viability V3 (10.92 log cfu/mL) showed a significantly higher viability than I4 (10.59 log cfu/mL). There was a significant increase in cell viability for both of the cultures upto 24 hrs indicating both cultures are good in term of viability but V3 isolate showed significantly higher viability than I4 isolate.

In a study, Mantzourani *et al*. (2018) used *Lactobacillus plantarum* ATCC 14917 as the starter culture to create fermented functional pomegranate juice. Cell viability was 11.42 0.16 log cfu/mL after fermentation, and it was 8.83 0.58 log cfu/mL after four weeks of storage at 4ºC.

Luca and Oroian (2021) used prebiotics *viz.* chicory inulin, soluble potato starch, oligofructose along with control in a study by to maintain an adequate quantity of microencapsulated probiotics. As a component of the encapsulation matrix, glucose, a carbon source, was employed. The produced microcapsules had noticeably diverse physical and chemical characteristics, as well as varying viability during preparation, storage, and imitated GI conditions. In relation to the size of the microcapsules and the method employed, the encapsulation efficiency was quite high (between 87.00 and 88.19%). After 10 hrs of incubation while combined with prebiotics, viability was discovered to be 9.52 to 9.64 log cfu/mL.

Overall, in the experimental conditions *Lactobacillus helveticus* MTCC 5463 (V3) culture showed better results with comparison to *Lacticaseibacillus rhamnosus* MTCC 5462 (I4). As per statistical analysis, V3 showed better growth profile than I4 at 24 hrs of incubation for phenol tolerance, cell viability, auto-aggregation assay, cell surface hydrophobicity. No significant change was observed for pH change of media and for bile and pancreatin tolerance I4 culture showed better results. As the statistical data indicates V3 culture is superior to that of I4 culture in terms of some functional properties can be considered as a better probiotic candidate for future use.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: KD: Involved in investigation, data generation, preparing original draft; KG: Engaged in conceptualization, data curation, supervision and final editing; MH, SH, AS: Involved in statistical analyses, methodology and editing.

Ethical statement: Work done in research paper was not involved any animal studies, hence there is no need for approval of the ethical committee.

Data availability statement: Research data regarding replication and statistical analysis was conducted and author have data related to results published in this research paper and will be made available on demand.

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