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In vitro INHIBITION OF PATHOGENIC BACTERIA *Helicobacter pylori* BY POTENTIAL PROBIOTIC BACTERIA *Lactobacillus casei* STRAINS

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ABSTRACT

Aim of these objectives the antimicrobial effects of *Lactobacillus casei* strains were tested against 3 strains of *H. pylori* were used well diffusion assays method and co-culturing of *Lactobacillus strain* and *H. pylori*. Antagonistic effect of six different *Lactobacillus* spp. was determined against one type strain of *H. pylori* 11638 and two *H. pylori* 21175, *H. pylori* 3119299, respectively. Six good *Lactobacillus* strains *L. casei* DCM 1008, *L. casei 16s MG3*, *L. casei BN11*, *L. casei MG3, L. casei H248* and *L. casei LBR1* were selected for additional assessment. The unadjusted and neutralized supernatants collected from all six *Lactobacillus* spp showed inhibition against all three *H pylori* isolates. *L. casei* DCM 1008, MG3, BN11, MG3, *LBR1* and H248 had shown better inhibition than the rest of *Lactobacillus casei* spp. Co-culturing of *Lactobacillus casei*, 16S isolate MG3, BN11 and H248 including all three *H. pylori* showed high inhibition against this pathogen. All strains of *Lactobacillus* produced adjustable concentrations of acetic acid and lactic acid.

Keywords: *H. pylori*; *Lactobacillus*; *Lactobacillus casei*; antagonistic.

INTRODUCTION

Lactic acid bacteria (LAB) are generally used in the manufacture of fermented foods and drinks that contribute to sensory qualities and food preservation and prevention. In addition, a large number of them are in ordinary human and animal gastrointestinal flora [1]. Benefits and utilization of lactic acid bacteria promote health well-known for several years, from [2]. Permission rage in Bulgaria Boerne for consumption of fermented milk [3]. The term 'probiotic' was first described as a live microbial nutritional supplement that beneficially affects the host by improving its innate microbial balance'. Some of the most well-known probiotics are related to the genus *Lactobacillus*. Some *Lactobacillus casei* and *Lactobacillus acidophilus* have been found to have probiotic properties, and these varieties have been used to treat gastrointestinal diseases. LAB showed bactericidal activity against some pathogens [4]. Many probiotics are now

commonly incorporated into food products such as fermented milk, yogurt and fermented or unfermented suns, and are marketed as freeze-dried supplements.

The human gastrointestinal microflora is a stable ecology under normal conditions in which microorganisms remain comparatively stable [5]. The function of normal microflora is not well known, but the two main functions of the host are resistance to colonization by diseases caused by pathogen-retention and the stage of certain metabolic functions. It is important to maintain the innate ecological flora to avoid pathogenic bacteria [6]. The widespread use of antibiotics has no single lead to an increase in the number of antibiotic-resistant pathogenic microorganisms but is often allied with alteration of the protective microflora, embarking on the occurrence of infections. Intended for these reasons, combating infection with an antibiotic-free approach is essential, and bacterial replacement therapy using organic microflora is a promising option. Protein microorganisms are living bacteria that work for the benefit of the host. It is generally accepted that these microorganisms can be useful tools in controlling the overgrowth of pathogen bacteria and consequently in preventing infections. Several *in vitro* and *in vivo* studies with different potential probiotic bacteria have demonstrated that these bacteria can interfere with viral growth and properties [7] and [8].

Helicobacter pylori is a gram-negative and spiral shaped bacterium, a microaerophilic gastric pathogen bacterium that infects higher than 50% of the world's population [1]. The first was isolated in 1984 in gastric biopsy samples from patients suffering from gastritis and peptic ulcers. After their discovery, researchers around the world confirmed the existence of these organisms in the gastric mucosa. It is clear that *H pylori*, normally for life with antimicrobial treatment. Some authors have reported the antagonistic activity of *Lactobacillus* against *H pylori* [9].

The LAB strain of *L. acidophilus* which administers an antimicrobial agent and found that natural culture used decreased the viability of the *H. pylori* strain in invitro experiment [10]. The authors noted that intestinal obstruction is caused by *H. felis* in mice [1]. *In vitro* inhibition of *H. pylori* depends on the ability of *L. casei* Shirota cells. The antibiotic activity of potential probiotic bacteria has been attributed to antibiotic-induced antibiotics [11]. These include many metabolites, pure-organic acids, and bactericides. Some studies have also shown that the acid produced by these bacteria is responsible for the inhibition of *H. pylori* [12]. Probiotic agents can play an important role [13].

The aim of the study was antimicrobial effects of *Lactobacillus* was tested against 3 patient strains of *H. pylori* using co-culturing of Lactobacillus and *H. pylori* and diffusion assays.

MATERIALS AND METHODS

The *H pylori* were grown on blood agar at 35° C for 72 h under the microaerophilic condition. Sub-culture was prepared on a similar agar for 72 h at 35° C and the microorganisms were preserved by maintaining at -80 °C in blood broth.

Selection of Bacterial Strain

22 strains of *Lactobacillus* strains of the *Lactobacillus casei* group were received from **Jayoti Vidyapeeth women's university Jaipur State of Rajasthan** will be used for isolating LAB probiotic bacteria. The organisms were activated by growing two times in MRS broth at 37°C for twenty-four h. The *Heliobacter pylori* used in this research included one variety of strain ATCC *H. pylori* 11638, patients isolate *H. pylori* Fiona 21175 and 3119299 each one was grown in BHI broth under anaerobic conditions for 72 hours at 35 °C.

Growth Inhibition of *H. pylori* with *Lactobacillus*

The samples of *lactobacillus casei* 22 strains were cultured at 37 °C for 24 hours by using 1% inoculums two to three times. To obtain a supernatant, 200 ml aliquots of the all 22 *lactobacillus casei* strains were centrifuged at 3838 x g for 15 minutes at the temperature of 4 °C. The supernatant was divided into two different parts in test tubes. The pH of the first part was 07.0 using 2N NaOH. The pH was measured at

20-22 ° C using a pH meter once regulated with pH 7.0 and 4.0 buffers. The pH of the second part was recorded and non-adjusted, each supernatant was filtered using 0.45 µm Millipore filters (Millipore Corp., Bedford, MA, USA). All three *Helicobacter pylori* strains were prepared by spreading 0.5 milliliters of culture on BHI agar plates. Each plate was 4 quartered and the wells were cut in the agar using a sterile 6 mm punch hole. The lowest of each well was wrapped with sterile 0.9% agar and then 100 µl of supernatant from each *Lactobacillus casei* strain was added. All inhibition assays were performed under aerobic conditions three times for each pathogenic microorganism, for highly adapted and unabated. The supernatant was diffuse for 2 hours into agar and incubated at 35 °C for 72 hours.

The Effect of Growing *H pylori* (Supernatants Obtained from *Lactobacillus* Strains*)*

The cultures were activated with 3 successive transitions and all supernatants were obtained as discussed above. The supernatants were divided into two different parts, the first part was not adjusted and the rest was adjusted to (pH 7.0) with 5N NaOH solution. 1 milliliter of the highly neutralized supernatant 1 ml of *H. pylori* type strain 11637 and the other two patients isolates 21174 and *H. pylori* 3119299 were placed in McCartney flasks with 10 ml of BHI broth, incubated at 35.°C for 24 hours. The control consisted of 10 ml of BHI broth, 1 ml of distilled water and 1 ml of all pathogenic bacteria. Growth inhibition of *Helicobacter pylori* was observed by measuring visual density using a (Nova space II spectrophotometer Pharmacia, Biotech, UK) at 620 nm for 12 hours for 72 hours.

The Effect of Growing *H. pylori* and *Lactobacillus* Strain in the Existence of Supernatant Obtained from *Lactobacillus*

The effects of each Lactobacillus supernatant on *H. pylori* growth were determined by adding 1 mL of each of the seven *Lactobacillus* supernatants to a 1 mL aliquot of *H. pylori* in 10 mL BHI broth, after that incubated at 35 °C for 72 hours, instead of supernatant, 1 mL of distilled water was used as a control. To measure (OD) optical density, portions were removed at 0, 25, 47, and 71 hours, and *H pylori* were calculated using BHI agar. Under anaerobic circumstances, each plate was incubated for 72 hours at 35 °C.

Co-culturing of *Lactobacillus* and Selected Pathogens in (BHI Broth)

The co-culture of the 7 periods *Lactobacillus* with 2 *H. pylori* was independently assessed by mixing 1 ml of each in 10 ml of BHI broth and the common cultures were grown under anaerobic condition for 72 hours. In the control instead of *Lactobacillus* cells. 1 ml aliquot was obtained at 12-hour intervals to measure pH, optical density, and count of each group of bacteria.

Lactobacillus was calculated by using the pour plate method in MRS agar containing 0.1% vancomycin. The plates were incubated under aerobic conditions at 37 °C for 72 h. BHI agar was used for *H pylori* count and the plates were stolen for 72 h anaerobically at 35 °C.

Activity determination

The activity of each *Lactobacillus casei* strain was determined by measuring the last fermentation product such as (lactic acid and acetic acid) by high-performance liquid chromatography. Each *Lactobacillus casei* strain was grown in MRS broth. One hundred microelements of (15.8 M HNO_3) and (14.9 ml 9 mM H_2SO_4 9 mM) were added to a 1.5 ml sample and centrifuged at (4000 xg) for 10 minutes using a benchtop centrifuge. Two milliliters of aliquot were stored at -20 °C until analysis. For the analysis of organic acids, the Aminex HP87H column with disposable cartridges at 65°C were used. The degassed mobile phase of H_2SO_4 9 mM, filtered over a 0.45 µm membrane filter (Millipore), was used with a movement rate of 0.3 ml/min. The detection wavelength was optimized at 220 nm to quantify organic acid. Standard solution of organic acid in the mobile phase to establish elution instances and calibration curves. The retention instances and the usual coefficient for acetic acid and lactic acid were 16.2 and 25.7 minutes and 0.9992 and 0.99947, respectively.

RESULTS AND DISCUSSION

Growth Inhibition of *H. pylori* with *Lactobacillus*

The growth inhibition of 3 *Helicobacter pylori* strains with 22 individual strains of the *Lactobacillus* group is presented in (Table 1). Commonly, the zones of inhibitions were greater with the addition of supernatants strains of *Lactobacillus durans* DCM 1008 16S, *Lactobacillus casei* 16S isolate MG3, *Lactobacillus casei* stress BN11, *Lactobacillus casei* 16S isolate MG3, *Lactobacillus casei* H248, *Lactobacillus acidophilus* LBR1 respectively. All the strains were used for further research. *H pylori*. The antimicrobial effects of *Lactobacillus* are a decrease in pH resulting from the production of lactic acids and acetic acids. The final pH of the unadjusted supernatant decreased from 6.4 at 0H to pH 3.7 - 4.02 at 24 h depending on the individual *Lactobacillus* strain (Table 1). We selected as earlier described seven robust *Lactobacillus* strains for further research.

Effect of Growing *H. pylori* with *Lactobacillus* Supernatants

The suppression of growth of 3 different major strains of *H. pylori* with six *Lactobacillus casei* supernatants as measured by a reduction in optical density is shown in (Table 1 & 2). In general, the 3 pathogens were considerably inhibited when supernatants of *Lactobacillus* strains grew over 48 hours as indicated by the decrease in optical density.

The addition of unadjusted supernatants of all six *Lactobacillus* strains resulted in greater suppression against pathogenic bacteria. While pH-balanced supernatants of, *L. casei* (DCM 1008 16S) 3.8, *L. casei* (16S isolate MG3), 3.89 for *L. casei* (BN11), 3.92 for *L. casei* (MG3) and 3.87 for *L. casei* (H248) 3.95 for (LBR1) *16S 3.88*. showed considerable inhibition (10-34%) against pathogenic bacteria, while pH-balanced supernatants of *Lactobacillus casei*, 16S isolate MG3, BN11, and MG3, H248 revealed significant inhibition 10- 34% against pathogenic bacteria.

Table 1. Inhibition of *H. pylori* with 22 *Lactobacillus* strains (supernatant)

Organisms List	pH	*H. pylori* (11637)	Patient isolate Fiona 21174	
		Zone of inhibition (mm)[1,2]		
Lactobacillus durans DCM 1008 16S	3.8	13.08 ± 0.9	13.22 ± 1.1	13.4 ± 1.0
BN11 16S	3.92	12.02 ± 0.8	12.11 ± 1.1	11.89 ± 0.7
DSM 9508 16S	4.01	11.85 ± 0.4	11.58 ± 0.5	11.62 ± 0.8
16S isolate MG3	3.89	12.33 ± 0.8	12.42 ± 0.9	12.75 ± 0.5
Y8 16S	3.92	13.2 ± 0.6	12.9 ± 0.9	12.55 ± 0.4
NLAE zl-P160 16S	3.96	10.1 ± 0.2	10.3 ± 0.6	10 .0 ± 0.8
E064 16S	4.03	9.8 ± 0.8	9.7 ± 0.4	9.2 ± 0.2
S-P155 16S	3.86	9.8 ± 0.8	9.7 ± 0.4	9.3 ±0.6
H248 16S	3.95	10.22 ± 0.8	11.44 ±1.3	11.25 ± 1.1
LBR1 16S	3.88	11.22 ± 0.4	10.92 ± 0.6	8.5 ± 0.2
CPI 10426 16S	3.9	7.75 ± 1.0	7.67 ± 0.9	7.50 ± 0.6
***L. casei* (MG3)**	3.87	8.17 ± 1.3	8.11 ± 0.6	8.33 ± 1.4
AVVO	4.02	9.00 ± 1.6	9.1 ± 2.0	9.8 ± 0.8
RACI5	3.81	10.0 ± 1.8 7	7.11 ± 0.6	7.92 ± 0.5
TKM W-231	3.87	12.01 ± 0.5	10.6 ±0.8	12.10 ± 0.2
Ke4I2C	3.9	10.8 ± 0.2	10.25 ± 0.2	10.15 ±1.1
16SrRNAgene, isolate 21	3.94	7.99 ± 1.1	7.82 ± 1.4	7.67 ± 0.9
mixed cultureTR	3.81	8.00 ± 0.9	8.17 ± 0.8	7.95 ± 1.1
strainFtrT22922	3.89	7.02 ± 1.05	7.59 ± 1.6	7.83 ± 1.2
strain33AS	3.88	12.25 ± 0.2	12.01 ± 0.4	12.69 ± 0.8
Enterococcus faecium spp	3.89	10.31 ± 0.9	10.83 ± 0.41	9.00 ± 1.0
faecium RDFTY	3.88	8.33 ± 1.4	8.67 ± 0.5	8.12 ± 0.9

1. The results are the means and standard variations of two independent experiments (n=6).
2. The zone of inhibition carries a (6mm) bore diameter.
3. Incubation condition: (CO_2 / 72 hours/ 35 degree).

Table 2. Effect of growing *H. pylori* with *Lactobacillus* supernatants

Optical density at 620 nm 2		Growth of *Lactobacillus* strains with Supernatant obtained from *H. pylori*					
	Control	*L.casei* DCM 1008		*16S isolate MG3*		*L. casei (BN11)*	
		Adj3	Undaj4	Adj3	Undaj4	Adj3	Undaj4
H. pylori (11638)	0.88	0.72	0.62	0.82	0.42	0.56	0.5
H. pylori (21175)	0.9	0.78	0.52	0.88	0.46	0.6	0.39
H. pylori (3119299)	0.98	0.81	0.47	0.92	0.59	0.64	0.41

	Control	*L.casei MG3*		*L.casei H248*		*L.casei LBR1*	
		Adj3	Undaj4	Adj3	Undaj4	Adj3	Undaj4
H. pylori (11638)	0.85	0.44	0.78	0.41	0.42	0.89	0.45
H. pylori (21175)	0.8	0.59	0.7	0.43	0.46	0.87	0.55
H. pylori (3119299)	0.91	0.64	0.63	0.37	0.59	0.91	0.67

Table 3.

	OD at 620 nm 2		Lactobacillus strains					
H. pylori	Bacterial count	Control	*L.casei* DCM 1008		*Stress MG3*		*BN11*	
			Adj3	Undaj4	Adj3	Undaj4	Adj3	Undaj4
(11638)	OD at $72h^1$	0.9	0.62	0.51	0.56	0.5	0.61	0.48
	CFU at $72h_2$	1.11×10^{10}	8.21×10^{10}	5.95×10^{10}	5.21×10^{8}	3.21×10^{6}	1.21×10^{8}	2.02×10^{8}
(21175)	OD at $72h^1$	0.92	0.72	0.41	0.6	0.39	0.58	0.31
	CFU at $72h_2$	1.21×10^{10}	1.35×10^{10}	8.65×10^{6}	4.25×10^{8}	3.68×10^{6}	7.42×10^{8}	2.58×10^{8}
(3119299)	OD at $72h^1$	1.12	0.82	0.62	0.94	0.61	0.88	0.51
	CFU at $72h_2$	1.80×10^{10}	5.55×10^{10}	7.23×10^{6}	6.12×10^{8}	3.91×10^{6}	5.551×10^{8}	1.90×108

	MG3		*H248*		*LBR1*	
	Adj3	Undaj4	Adj3	Undaj4	Adj3	Undaj4
(11638)	0.86	0.32	0.71	0.34	0.78	0.41
	4.21×10^{9}	1.02×10^{7}	5.12×10^{8}	1.45×10^{7}	422×10^{9}	9.92×10^{7}
(21175)	0.65	0.049	0.77	0.38	0.7	0.42
	3.35×10^{9}	4.28×10^{7}	6.68×10^{8}	1.25×10^{7}	5.85×10^{9}	1.02×10^{7}
(3119299)	0.71	0.39	0.74	0.38	0.51	0.27
	5.22×10^{9}	3.64×10^{7}	8.85×10^{8}	1.14×10^{7}	6.12×10^{9}	8.89×10^{7}

The results reveal that all of the *Lactobacillus casei* strains indicated a greater zone of inhibition. For *L. casei* (DCM 1008 16S) 3.8, *L. casei* (16S isolate MG3) 3.89, the pH of the supernatant was 3.90, 3.92for *L. casei* (BN11), 3.87 for *L. casei* (MG3), and 3.87, (LBR1) *16S 3.88 L. casei* (H248) 3.95.

In optical density, percentages are different between control and pathogens with supernatant obtained from Lactobacilli.

OD measured at a wavelength of 620 nm, pH 7, Left unadjusted.

The Effect of Growing *H pylori* and *Lactobacillus* Strain in the Existence of Supernatant Obtained from *Lactobacillus*

The effect of growing *h. pylori* with supernatant obtained from bacteria strains (*L. durans* DCM 1008, *L. casei* 16S MG3, *L. casei* BN11, *L. casei* MG3, *L. casei* H248, *L. acidophilus* LBR1, *L. casei*) are mixed as measured by optical density. bacterial counts are presented in **(Table 3)** In general, there was a decrease in optical density at 72 hours. A related trend was observed with bacterial counts. The inhibition of H pylori was vital ($p< 0.5$) whereas the supernatant was not

Table 4. Effect of co-culturing *Lactobacillus* and selected pathogenic bacteria

	OD $_{620}$2	CFU[4]	pH	OD 620	OD% Difference	CFU	CFU% difference	pH	pH difference
	L. casei DCM 1008								
***H. pylori* (11638)**	1.44	1.32×10^9	6.12	1.13	-21%	3.21×10^8	-76%	4.44	-1.68
***H. pylori* (21175)**	1.01	1.32×10^9	6.01	0.91	-9%	6.61×10^8	-50%	4.52	-1.49
***H. pylori* (3119299)**	1.29	9.95×10^9	6.22	.95	-26%	5.58×10^8	-94%	4.62	-1.6
	***L. casei* MG3**								
***H. pylori* (11638)**	1.44	1.32×10^9	6.12	1.59	11%	4.25×10^8	-68%	4.58	-1.54
***H. pylori* (21175)**	1.01	1.32×10^9	6.01	1.01	0%	8.88×10^8	-33%	4.42	-1.59
***H. pylori* (3119299)**	1.29	9.95×10^9	6.22	1.12	-11%	5.25×10^8	-95%	4.80	-1.42
	***L. casei* BN11**								
***H. pylori* (11638)**	1.44	1.32×10^9	6.12	1.69	18%	6.38×10^8	-52%	4.21	-1.91
***H. pylori* (21175)**	1.01	1.32×10^9	6.01	0.89	-11%	7.75×10^8	-41%	4.50	-1.51
***H. pylori* (3119299)**	1.29	9.95×10^9	6.22	1.29	0%	9.18×10^8	-91%	4.71	-1.51
	L. casei H248								
***H. pylori* (11638)**	1.44	1.32×10^9	6.12	1.55	8%	4.25×10^8	-68%	4.32	-1.8
***H. pylori* (21175)**	1.01	1.32×10^9	6.01	0.92	-8%	5.28×10^8	-60%	4.49	-1.52
***H. pylori* (3119299)**	1.29	9.95×10^9	6.22	1.30	1%	6.28×10^8	-94%	4.63	-1.59

Table 5.

Lactobacillus casei strains	**Acetic acid (mg/L)[1]**	**Lactic acid (mg/L)[1]**
L. casei DCM 1008	76.4 ± 2.1	1439 ± 73
L. casei stress MG3	117.6 ± 4.8	1350 ± 26
L. casei BN11	105.9 ± 3.1	1056 ± 31
*L. case i*MG3	97.6 ± 2.1	1245 ± 21
L. casei H248	51.7 ± 1.7	1457 ± 5
L. casei LBR1	31.1 ± 0.5	1464 ± 30

Data are the means and standard deviations of 3 independent tests (n=6)

neutralized, once supernatant was neutralized, the optical density readings were additionally significant ($p< 0.5$) for Lactobacillus (*L. durans* DCM 1008, *L. casei* 16S MG3, *L. casei* BN11, *L. casei* MG3, *L. acidophilus* LBR1, *L. casei* H248) (Table 3).

Effect of Co-culturing *Lactobacillus* and Selected Pathogenic Bacteria

The effect of co-culturing *Lactobacillus* spp (*Lactobacillus casei*, 16S isolate MG3, BN11, and MG3, H248) and pathogenic bacteria *H. pylori* is presented in (Table 4) respectively. There was a reduction in cell density when pathogens were co-cultured with *Lactobacillus casei* strains. Similarly, the pH was reduced by 1.43 to 1.91 log units when *Lactobacillus* was added with the bacteria of *H. pylori*. All pathogenic bacteria were significantly reduced with the addition of the above-mentioned *Lactobacillus.* The reduction ranges from (32.7 - 94.7%) with a decrease in pH between (1.42 - 1.91).

Activity Determination of *Lactobacillus*

One group of lactobacilli organisms includes a heterogeneous fermentation capacity that produces lactic acid and acetic acids as end products of glycolysis [4]. This is a major characteristic of *Lactobacillus*. As reported in (Table 5) *Lactobacillus casei* provided the highest level of acetic acid *L. casei* DCM 1008 (acetic acid 76.4 ± 2.1 and lactic acid 1439 ± 73), (117.6 ± 4.8) MG3, isolated with 16S and *L. casei* H248 produced the lowest level (31.1 ± 0.5). *L. casei* MG3 produced the highest level of lactic acid (1464 ± 30) and *L. casei* BN11 produced the lowest level (1056 ± 3.1). was produced by *L. casei* LBR1. The highest level of lactic acid (1464 ± 30) was produced by *L. casei* LBR1 while the lowest level (1056 ± 3.1) was produced by*L. casei* BN11 (Table 5).

CONCLUSION

Lactobacillus durans DCM 1008 16S, *Lactobacillus casei* 16S isolate MG3, *Lactobacillus casei* stress BN11, *Lactobacillus casei* MG3, *Lactobacillus casei* H248, *Lactobacillus acidophilus* LBR1, and *Lactobacillus acidophilus* LBR1 were all found to inhibit pathogenic and putrefactive microorganisms in inhibition assay methods and during growth with supernatants. The *Lactobacillus* strains' low pH could be responsible for the suppression of pathogenic and putrefactive bacteria. The inhibition of growth and zones was attributable to unknown antibacterial compounds produced by probiotic bacteria when the pH of the supernatant was adjusted to natural. These strains showed promise against *Helicobacter pylori*, the bacteria that causes peptic ulcers. To confirm *Lactobacillus* strains' antibacterial capabilities, more *in vivo* research is needed.

The result of co-culturing H pylori with supernatant obtained from *Lactobacillus* casei strains such as (*Lactobacillus* spp *Lactobacillus durans* DCM 1008 16S, *Lactobacillus casei* 16S isolate MG3, *Lactobacillus casei* stress BN11, *Lactobacillus casei* 16S isolate MG3, *Lactobacillus casei* H248, *Lactobacillus acidophilus* LBR1, are mixed and measured by (OD) optical density, they showed a similar decrease in the number of bacteria after 72 hours. The effect of co-culture was also investigated with *Lactobacillus* spp. (*Lactobacillus casei*, 16S isolate MG3, BN11 and MG3, H248) showed a reduction in cell density. Similarly, the pH dropped from (1.42 to 1.91) log units. All pathogenic bacteria were significantly reduced

with the addition of the mentioned *Lactobacillus* strains. These strains showed promising results against the *Helicobacter pylori*-causing ulcer. Further *in vivo* studies are required to confirm the antimicrobial properties of *Lactobacillus* strains.

FUTURE RESEARCH DIRECTIONS

In the developed world, lactic acid bacteria are associated with fermented dairy products such as yogurt, buttermilk and cheese. Widespread use of lactic acid bacteria has been put in place this century not only as an early culture is also associated with useful health effects. Today, an increasing number of healthy foods are also known as functional foods pharmaceutical products are promoted with specific health claims some types of lactic acid bacteria. By increasing the knowledge of potential probiotic bacteria and using it with various prebiotics around the world, the demand for products containing potential probiotic bacteria has been greatly developed. There has been an increase in the use of probiotic products made from dairy sources, such as fermented milk, yogurt, and other dairy products, as well as capsules or tablets containing freeze-dried probiotic organisms, due to larger bacterial densities.

Further research on the fermentation of different types of prebiotics by probiotic bacteria is critical to find out which products produce the most synbiotic response. Research into the mechanisms that explain why some prebiotics are more beneficial to certain probiotic strains than others would help us better understand the reactions that occur inside the gastrointestinal system.

The ability of probiotic microorganisms to inhibit *Helicobacter pylori*, particularly lactobacilli, has important clinical significance. Because many bacterial species become more increasingly resistant to antibiotics, probiotic research is critical to demonstrating their efficiency in reducing these strains from the human stomach. Our work has revealed that some Lactobacillus strains can successfully inhibit Helicobacter pylori *in vitro*. These strains require to be additionally assessed and critically evaluated by clinical examination on subjects colonised and infected with *Helicobacter pylori*. It may be helpful to examine which potential probiotic strains combined with prebiotics are most successful in reducing *H. pylori in vivo*. Without the utilization of antibiotics, which disrupt the balance of the gastrointestinal microflora.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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