

Probiotic Potentiality of *Lactobacillus coryniformis* subsp. *Torquens* MTi1 and *Lactobacillus coryniformis* MTi2 Isolated from Intestine of Nile Tilapia: An *In vitro* Evaluation

Md. Mijanur Rahman^{1*}, Salma Aktar¹, Mohammad Omar Faruk³,
Mohammad Sharif Uddin², Jannatul Ferdouse² and Md. Nural Anwar²

¹Department of Microbiology, Noakhali Science and Technology University, Noakhali - 3814, Bangladesh.

²Department of Microbiology, University of Chittagong, Chittagong - 4331, Bangladesh.

³Department of Statistics, Noakhali Science and Technology University, Noakhali - 3814, Bangladesh.

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Inefficiency of antibiotics because of emerging resistance, to reduce use of chemical preservatives and to alleviate other gastrointestinal or urogenital disorders- using probiotics get prioritized in recent times. We investigated probiotic potentiality of *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2 isolated from Nile Tilapia. A set of in vitro probiotic parameters (antibacterial activity, temperature sensitivity, pH, bile salt, NaCl tolerance, antibiotics resistance) were evaluated by performing disc diffusion, agar well diffusion methods and several other microbiological tests. Both isolates demonstrated antibacterial activity against *Escherichia coli*, *Salmonella Typhii*, *Bacillus subtilis* and *Pseudomonas aeruginosa* whereas *L. coryniformis* MTi1 also active against *Staphylococcus aureus*. They could grow at wide range of temperatures(27-45°C) and pH (2-9); had tolerance to 0.5-2% bile salt and 1-7% NaCl; ferment lactose. *L. coryniformis* subsp. *torquens* MTi1 was not killed by Cefixime(5µg) whereas *L. coryniformis* MTi2 neither killed by Cefixime(5µg) nor Streptomycin(10µg) in antibiotic susceptibility testing. Best antibacterial production of isolates found at 37°C, pH 2 and after 72 hours of incubation. We observed approximately 42% and 40% more antibacterial production from *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2 respectively after 72 hours of incubation instead of 24 hours. *L. coryniformis* MTi2 could produce bacteriocin or bacteriocin like substances active against *E. coli* (15mm) and *B. subtilis* (14mm). The study concludes based on empirical observations that, the isolates have potentiality to be efficient probiotic which will eventually contribute to promote public health.

Keywords: Probiotics, *Lactobacillus coryniformis*, Nile Tilapia, Antibacterial activity.

Probiotics are widely recognized as helpful bacteria as they play role to keep our gut healthy. FAO and WHO (2001) defined probiotic as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host”¹. Injudicious or haphazard use of antibiotics to challenge frightful pathogens, extensive application of chemical preservatives to preserve foods and food products and increase

in the prevalence of allergic diseases due to environmental hazards have imposed a great threat to human health². To overcome those challenges, scientists and regulatory authorities are looking for alternative living agents and thus probiotics get prioritized.

Probiotics have been used to reduce the duration of infectious diarrhea, shortening the initial phase of watery stool, increase the amount of IgA antibody and decrease virus shedding in viral diarrhea³. Probiotics have also been reported to control lactose intolerance, irritable bowel syndrome, and reduction of gastric

* To whom all correspondence should be addressed.

Tel.: +88 01823103818;

E-mail: mijan.cu91@gmail.com

ulcers^{4,5}. To balance the disequilibrium in the immune response causing the allergic response and to reduce the levels of blood cholesterol by consumption of probiotics containing dairy foods have also been demonstrated^{2,6}. To substitute the employment of antibiotics which are used to maintain a good balance of the gastrointestinal tract (GIT) microflora, to eliminate pathogenic microorganisms, to facilitate the reduction of gastrointestinal upsets probiotics are proven to be effective⁷. *Lactobacillus* spp. are ubiquitous in nature, generally regarded as safe and most often used as probiotics. There are several salient features behind its popularity: lactobacilli are found naturally in the human gut; particularly common in the small bowel; grow in the absence of oxygen or small amounts of oxygen; they feed sugars as a source of carbon which are plenty in the intestine; require a range of minor nutrients to enable them to live and grow, as there is a wide range of food in that part of the intestine so they are prominent there. Besides intestine, they are also found in the vagina where they are the dominant resident bacteria and they protect against vaginal infection^{8,9}. *Lactobacillus coryniformis*, have been studied for its potential probiotic efficiency. *L. coryniformis* CECT 5711 has the ability to produce both reuterin and cobalamin¹⁰. It has been found to survive when exposed to conditions found in the gastrointestinal tract, and particularly low pH and bile, which seems to be a critical pre-requisite for a probiotic strain¹⁰. The strain seemed to adhere to the intestinal mucosa, a pre-requisite for gut colonization that may lead to the competitive exclusion of pathogenic bacteria, displayed β -galactosidase activity that can exert beneficial effects in the host¹¹. *L. coryniformis* have been reported to modulate the immune response against allergic reactions. Study conducted by Martinez *et al.* (2009) showed that the consumption of a probiotic product containing *L. coryniformis* CECT5711 along with *L. gasseri* CECT5714 induced beneficial effects on immunological parameters in allergic children². Obesity is associated with intestine dysbiosis and is characterized by a low-grade inflammatory status, which affects vascular function. Animal model experiment revealed that an endothelial-protective effect of probiotic *L. coryniformis* CECT5711 in obese mice by increasing NO bioavailability,

suggesting the therapeutic potential of this gut microbiota manipulation to prevent vasculopathy in obesity¹².

Considering those facts and discussed perspectives, this study focused on isolation of *Lactobacillus* spp., from intestine of Nile Tilapia (*Oreochromis niloticus*) fishes and *in vitro* evaluation of their probiotic potentiality.

MATERIALS AND METHODS

Sample collection

Healthy Nile Tilapia (*Oreochromis niloticus*) fishes were collected from local ponds in Chittagong region, Bangladesh. The fishes were washed thoroughly with sterilized distilled water and processed in lab within two hours of collection.

Isolation, selection and identification of *Lactobacillus* spp.

For isolation of *Lactobacillus*, selective media for Lactic acid bacteria (LAB), MRS (De Man Rogosa Sharpe) was used and serial dilution agar plate technique was implemented. About 10g of fish intestine was cut aseptically and homogenized with 90 mL normal saline in a homogenizer and serially diluted up to 10^{-5} . About 1 mL of each dilution of the sample was pipetted into sterile petri plates. MRS agar media were poured and incubated at 37°C for 24-48 hours¹³.

As probiotic *Lactobacillus* should be good antimicrobial producer, the isolated *Lactobacillus* were first screened for antibacterial activity against five selected test organisms. 200mL of MRS broth was autoclaved at 121°C for 15 minutes and inoculated with the colonies *Lactobacillus* and incubated at 37°C for 2-3 days under stationary condition. Then it was centrifuged at 9000 rpm for 15 minutes at 4°C. The supernatant was then filtered through Whatman No. 1 filter paper to remove residual cells. Petri-plates were prepared by pouring sterile molten Mueller Hinton medium and allowed it to solidify. 100 μ L of each standardized test microorganisms were spread on agar plates. Two wells (each 7mm in diameter) made into agar plates with sterile borer. The wells were loaded with 100 μ L of filtered cell free supernatant (CFS) and 100 μ L sterile broth. Plates were incubated at 37°C for 24 hours. After incubation, diameter of zone of inhibition was observed and measured¹⁴.

Finally, the good antibacterial

producer *Lactobacillus* spp. were identified by characterization on the basis of their morphological characteristics including size and shape of the organism, arrangement of the cells, presence or absence of the spores, regular or irregular forms, acid fastness, gram reaction etc.; cultural and physiological characteristics including H₂S production, nitrate reduction, deep glucose agar test, fermentation of different carbohydrates etc. All these characteristics were then compared with the standard description of “Bergey’s Manual of Determinative Bacteriology”, 8th edition¹⁵.

Test microorganisms

The test pathogenic organisms used in this study were *Escherichia coli* ATCC25922, *Salmonella* Typhii AE14296, *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* IFSTIM22, *Pseudomonas aeruginosa* CRL(ICDDR,B). The test microorganisms were standardized by using 0.5 Mc Farland standard solutions which gives approximate cell density of 1.5×10^8 CFU/mL, having absorbance of 0.132 at wavelength of 600nm¹⁶.

Assessment of in vitro potentiality of *Lactobacillus* spp. based on evaluating following parameters

Temperature sensitivity

Lactobacillus spp. separately inoculated in MRS broth was incubated at varying temperatures, i.e., 27, 37, and 45°C for 24-48 hours. After incubation, the absorbance of MRS broths were taken at 600nm by a spectrophotometer to measure microbial load.

pH tolerance

As like above experiment, the *Lactobacillus* cultures were separately inoculated into sterile MRS broth tubes of varying pH (2, 4, 7 and 9) incubated at 37°C for 24-48 hours. The absorbance of MRS broths was taken at 600nm by a spectrophotometer to measure microbial load.

Bile salt tolerance

The MRS broth media with different concentrations of bile salt (0.5, 1.0 and 2.0%) were inoculated separately with each *Lactobacillus* sp. and incubated at 37°C for 48 hours. Then the absorbances of MRS broths were taken at 600nm by a spectrophotometer for measuring microbial load.

NaCl tolerance

The MRS broth media prepared with

varying concentrations of NaCl (1, 3 and 7%) were inoculated separately with each *Lactobacillus* sp. and incubated at 37°C for 48hours. Then the absorbances of MRS broths were taken at 600nm by a spectrophotometer for measuring microbial load.

Response to some common antibiotics

Sensitivity of *Lactobacillus* spp. to some commonly used antibiotics was determined by antibiotic susceptibility testing performed according to Kirby-Bauer discs diffusion method on MRS agar plates. The used antibiotics were Penicillin G(10units), Chloramphenicol(30µg), Erythromycin(15µg), Cefixime(5µg), Cephadrine(30µg), Streptomycin(10µg) and Rifamycin(5µg).

Lactose utilization

The acid production by *Lactobacillus* spp. was detected by observing the change in color of the medium red to yellow. Sterilized fermentation medium (Peptone 10g, NaCl 15g, phenol red 0.018g, lactose 5g, for 1L distilled water and final pH 7.0) was inoculated with *Lactobacillus* cultures and incubated at 37°C for 24-48 hours. Change in color from red to yellow indicates the production of acid¹⁷.

Characterization of probiotic *Lactobacillus* spp. by determining optimum temperature, pH and incubation period for getting best antibacterial production

Optimum Temperature

To determine the optimum temperature for getting maximum antibacterial production by the *Lactobacillus* spp., MRS broth tubes were prepared for each isolate and inoculated with *Lactobacillus* sp. Then the MRS broth tubes were incubated at three different temperatures (27°C, 37°C and 45°C) for 24-48 hours. Then the culture broth was centrifuged at 9000rpm for 15minutes and filtered through Whatman No. 01 filter paper. Then antibacterial activity of the cell free supernatant (CFS) was assayed against respective test organism (previously showed antagonistic activity) by agar well diffusion method. (100µL CFS in each well)

Optimum pH

MRS broth were adjusted for four different pH (2, 4, 7, 9) using 0.1N HCl and 0.1N NaOH. Each *Lactobacillus* sp. was inoculated separately and incubated at optimized temperature for 24-48hours. Then the culture broth was centrifuged

at 9000rpm for 15minutes and filtered through Whatman No. 01 filter paper. Then antibacterial activity of the CFS was assayed against respective

test organism by agar well diffusion method. (100µL CFS in each well)

Optimum incubation period

MRS broth tubes were prepared and the pH was adjusted to optimum for each isolates. Each *Lactobacillus* sp. was inoculated separately and incubated at optimized temperature for 24, 48, 72 and 96hours. After completing each incubation period broth was centrifuged at 9000rpm for 15minutes and then filtered through Whatman No. 01 filter paper. Then antibacterial activity of the culture supernatant was assayed against respective test organism by agar well diffusion method. (100µL CFS in each well)

Determination of reasons behind antibacterial activity of *Lactobacillus* spp.

To determine cause(s) (Lactic acid/ acetic acids, H₂O₂ or Bactericins/Bacteriocin like substance) of antibacterial activity, 1mL of frozen *Lactobacillus* isolate was cultured overnight in

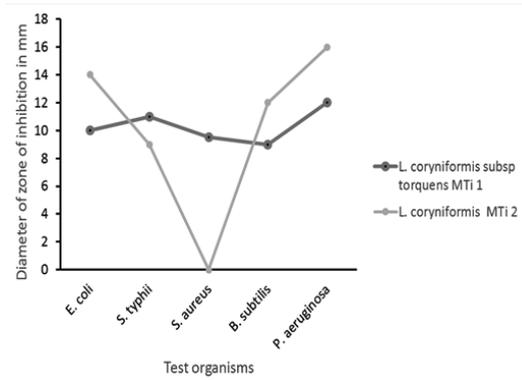


Fig. 1. Antibacterial activity of *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2 against five test organisms

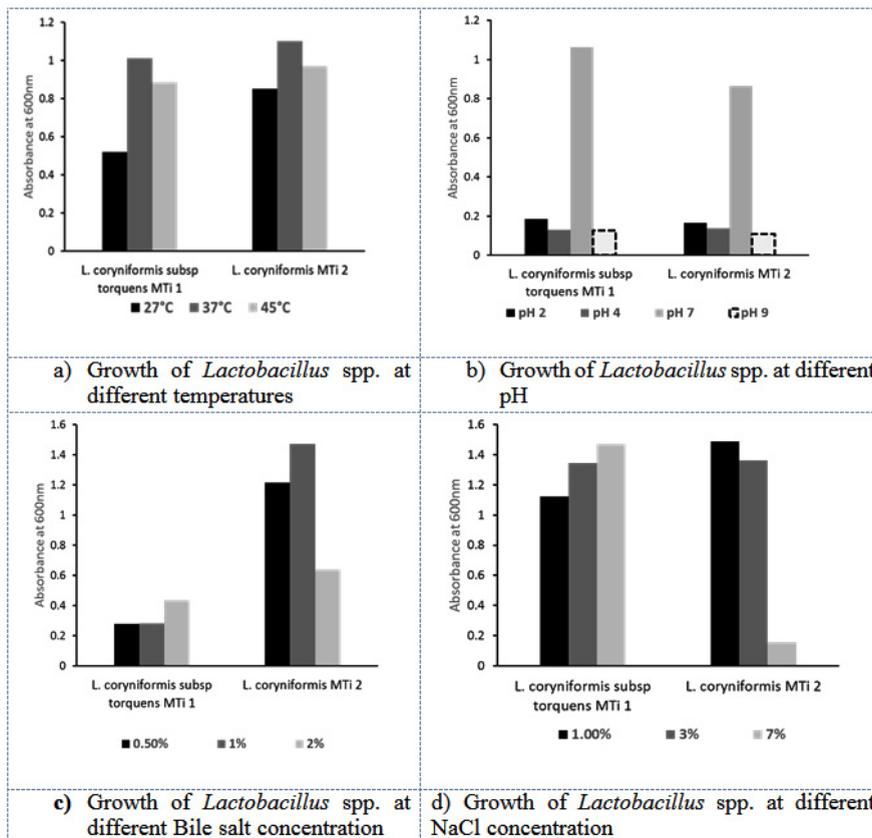


Fig. 2. (a-d). Both *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2 could withstand at wide range of temperatures(27-45°C), pH (2-9), bile salt concentration (0.5-2%) and NaCl (1-7%) treatment

20mL MRS broth. Then 1mL of this culture was sub-cultured overnight in 20mL fresh MRS broth. Cells were removed by centrifuging at 9000rpm for 15minutes. The supernatant was filtered through a sterile Whatman No. 1 filter paper and 100µL of the pH unadjusted aliquot of cell free supernatant (CFS) was added to the first well. The remaining CFS was adjusted to pH 6.0 with 1M/IN NaOH in order to rule out possible inhibitory effects due to organic acids. 100µL of the pH adjusted CFS was filtered and added to the second well. The neutralized CFS was then treated with 1mg/mL of catalase (Merck KGa A, Germany) at 25°C for 30min to eliminate the possible inhibitory action of H₂O₂ and filtered. Then 10µL catalase treated CFS was placed in the third well. If inhibition zone were found in the third well, the isolates were considered

to be able to produce bacteriocin or bacteriocin like substances)¹⁸.

RESULTS

Isolation, identification and selection of *Lactobacillus* spp.

Fifteen (15) strains of *Lactobacillus* spp. have been isolated from intestine of Nile Tilapia fish. Among them only 2(13%) isolates showed potential antibacterial activity against five test pathogenic organisms. Finally, the two isolates were identified as *Lactobacillus coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2 based on conventional methods using cultural, morphological characteristics and biochemical reactions as described in “Bergey’s Manual of Determinative Bacteriology”, 8th edition¹⁵.

Lactose utilization

The acid production by utilizing lactose in the medium by the selected *Lactobacillus* isolate was detected by observing the change in the color of the medium from red to yellow. Both isolates showed the capability of producing lactose in test medium.

Characterization of probiotic *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2

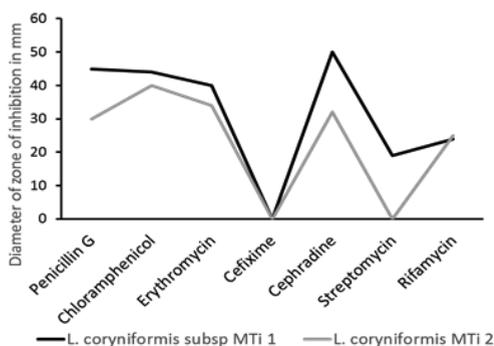


Fig. 3. Resistance pattern of *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2 against some commonly used antibiotics

DISCUSSION

The aim of this study was to isolate *Lactobacillus* spp. having potentiality to be used as probiotic from intestine of Nile Tilapia. To be

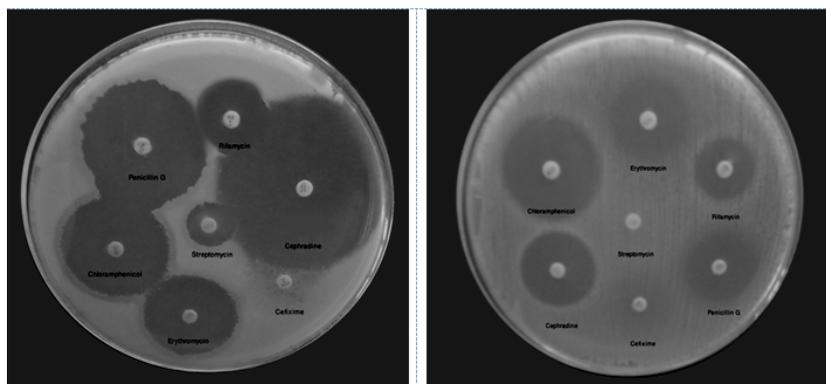


Fig. 4. Photograph of antibiotic susceptibility testing (AST) of *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2

an efficient probiotic, *Lactobacillus* spp. must have potential antimicrobial activity against gut pathogens, survival capability at intestinal pH and temperatures, could utilize lactose, tolerance to bile salt and NaCl; may have resistance to commonly used antibiotics. Our isolated *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2 demonstrated antibacterial activity against *E. coli*, *S. Typhii*, *B. subtilis* and *P. aeruginosa*. *L. coryniformis* MTi1 also additionally showed antibacterial activity against *S. aureus* (9.5mm). We observed that *L. coryniformis* subsp. *torquens* MTi1 showed better antibacterial activity against *S. Typhii* (11 vs 9mm) than *L. coryniformis* MTi2 while *L. coryniformis* MTi2 has comparatively greater antibacterial activity against *E. coli* (14 vs 10mm), *B. subtilis* (12 vs 9mm), *P. aeruginosa* (16 vs 12mm) (Fig. 1). Martin *et al.*, (2005) reported that *L. coryniformis* CECT 5711, a strain isolated from a goat's milk cheese, displayed a broad-spectrum antimicrobial activity; could produce lactic acid, acetic acid, reuterin and cobalamin,

a cofactor required for conversion of glycerol to 3-HPA through a glycerol dehydratase¹⁰. Nair and Surendran (2009) also reported that lactic acid bacteria isolated from fish and prawn have inhibitory activity against *Listeria monocytogenes*, *Bacillus cereus*, *E. coli*, *S. aureus* and *S. Typhii*¹⁹.

Both isolates could grow at wide range of temperatures (27-45°C) though best growth found at 37°C (Fig. 2a), and showed satisfactorily tolerance to wide range of pH (2-9) in vitro growth medium. Although they are grow less in pH 2, 4 or 9 but maximum growth found at neutral pH (7) (Fig. 2b). Resistance to low pH is an important selection criterion for probiotic microorganisms as gastric juice in the stomach destroys most microorganisms ingested. Many other studies showed that the exposure of *Lactobacillus* strains to pH values of 2.5–4.0 does not influence their survival rate, but it dropped at lower pH values. The ability of lactobacilli to survive the passage through media with physiological pH of 2-3 (to mimic the stomach environment) was reported to be variable

Table 1. Determination of optimum temperature, pH and incubation period for getting best antibacterial production from probiotic *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2

Antibacterial activity against selected test organisms	<i>L. coryniformis</i> subsp. <i>torquens</i> MTi1			<i>L. coryniformis</i> MTi2		
	Tem. (°C)	pH	Incubation time (Hours)	Temp. (°C)	pH	Incubation time (Hours)
Maximum Average	37	2	72	37	2	72
Least CV [#]	37	7	24	37	4	72

[#] CV is the Coefficient of Variation

Table 2. Determination of reasons behind antibacterial activity of *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2 by gradually eliminating antibacterial effect of acids and H₂O₂ (Diameter of zone of inhibition in mm)

	<i>E. coli</i>		<i>S. Typhii</i>		<i>P. aeruginosa</i>		<i>B. subtilis</i>		<i>S. aureus</i>	
	MTi1	MTi2	MTi1	MTi2	MTi1	MTi2	MTi1	MTi2	MTi1	MTi2
Raw CFS	17	23	17	20	24	22	18	24	19	–
pH adjusted CFS	10	19	12	15	17	11	12	19	18	–
pH adjusted and Catalase treated CFS	–	15	–	–	–	–	–	14	–	–

MTi1: *L. coryniformis* subsp. *torquens* MTi1; MTi2: *L. coryniformis* MTi2; CFS: Cell Free Supernatant. “–”: No antibacterial activity.

and strain dependent, but with a survival rate of approximately 85%, which is very significant for the probiotics²⁰.

In this study, *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2 were able to survive at 0.5 to 2% bile concentration in test media. The best growth of *L. coryniformis* subsp. *torquens* MTi1 found with 2% bile salt whereas *L. coryniformis* MTi2 at 1%. It is apparent that growth ability of *L. coryniformis* MTi2 is better than *L. coryniformis* subsp. *torquens* MTi1 in presence of bile salt (Fig. 2c). Tolerance to bile salts is a prerequisite for colonization and metabolic activity of bacteria in the small intestine of the host which eventually help Lactobacilli to reach the small intestine and colon and contribute in balancing the intestinal microflora²¹. Both isolates also showed their growth capability in presence of 1-7% NaCl in growth medium though best growth found with 3% NaCl. The cell density indicates that NaCl may have promoting effect on the growth of the *Lactobacillus* isolates (Fig. 2d). Hoque *et al.*, (2010) reported that *Lactobacillus* sp. isolated from yoghurts can tolerate 1–9% NaCl. Pundir *et al.*, (2013) also made similar observations regarding the NaCl tolerance of *Lactobacillus* spp¹⁴.

L. coryniformis subsp. *torquens* MTi1 and *L. coryniformis* MTi2 both could utilize lactose in growth medium which is another important selection criterion for probiotics. Lactose intolerant people cannot metabolize lactose due to the lack of essential enzyme α -galactosidase which resulting symptoms including abdominal pain, cramping and diarrhea when they consume milk or lactose containing products. If lactose passes through from the small intestine, it is converted to gas and acid in the large intestine by the colonic microflora. Other studies provide evidence that the addition of certain starter cultures (lactose utilizing *Lactobacillus* sp.) to milk products, allows the lactose intolerant people to consume those products without the usual rise of breath hydrogen or associated symptoms¹⁴. The oral administration of the probiotic strains combination of the probiotic strains *L. gasseri* CECT5714 and *L. coryniformis* CECT5711 led to an improvement of parameters such as the production of short chain fatty acids, the fecal moisture and the frequency and volume of the stools result a clear improvement in intestinal

environment eventually make positive effect on healthy adults^{22,23}.

Small scale antibiotic susceptibility testing revealed that both *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2 could survive against few commonly used antibiotics. We observed that *L. coryniformis* subsp. *torquens* MTi1 was not killed by Cefixime whereas *L. coryniformis* MTi2 neither killed by Cefixime (5 μ g) nor Streptomycin (10 μ g) (Fig. 3 and 4). Resistance of the probiotic strains to some antibiotics could be used for both preventive and therapeutic purposes in controlling intestinal infections; wide spectrum resistance of antibiotics indicated that if isolated probiotics induced in patients treated with antibiotic therapy may be helpful in faster recovery of the patients due to rapid establishment of desirable microbial flora²⁴. The above mentioned data and scientific facts indicate that our isolated *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2 showed potential characteristics to be efficient probiotic though it still requires further additional confirmatory *in vivo* experiments to be performed.

We also tried to optimize temperature, pH and incubation time to get the best antibacterial production by the *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2 against respective test pathogens (previously showed antagonistic activity). Cell free supernatant (CFS) of the isolate was tested for antibacterial activity against test organisms by agar well diffusion method after letting the isolates grow at different temperatures (27, 37 and 45°C). The maximum average and least Coefficient of Variation (CV) of antibacterial production of *L. coryniformis* subsp. *torquens* MTi1 against test pathogens found at 37°C. Hence, 37°C is the optimum temperature for the antibacterial production. Keeping optimum temperature constant, the maximum average of antibacterial production of *L. coryniformis* subsp. *torquens* MTi1 found at pH 2 but the least CV found at PH 7, which means that consistent antibacterial production of *L. coryniformis* subsp. *torquens* MTi1 occurred at PH 7 even the maximum average antibacterial production was at PH 2. By keeping temperature at 37°C and pH at 2, the maximum antibacterial production from *L. coryniformis* subsp. *torquens* MTi1 found after 72 hours though

the consistent antibacterial production against tested pathogens found just after 24 hours. Again, in case of *L. coryniformis* MTi2, the optimum temperature is found at 37°C since both maximum average and least CV of antibacterial production by the isolate found at 37°C. There is no optimum pH for best antibacterial production found for *L. coryniformis* MTi2 as maximum average found at pH 2 and least CV found at pH 4. The optimum incubation period for *L. coryniformis* MTi2 found after 72 hours as both maximum average and least CV of antibacterial production found after 72 hours (Table 1). After performing ANOVA (Analysis of Variance), the parameters temperature and incubation period for getting best antibacterial production by *L. coryniformis* subsp. *torquens* MTi1 are found to be significant ($p < 0.05$), i.e., with the change in temperature and incubation period a significant change will occur in antibacterial production. With changing of pH, the changes in antibacterial production by *L. coryniformis* subsp. *torquens* MTi1 is insignificant, i.e., the change in antimicrobial activities is very less considerable. On the other hand, in case of *L. coryniformis* MTi2, with changing in pH and incubation period, a significant ($P < 0.05$) change will occur in antibacterial production.

To determine which substance (Lactic acid/acetic acids, H_2O_2 , Bacteriocins or Bacteriocin like substances) cause the antibacterial activity, CFS of the *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2 were first neutralized using NaOH/HCl to diminish the antibacterial activity of acid, then treated with catalase enzyme to reduce antibacterial activity of H_2O_2 and then antibacterial activity of CFS assayed by agar well diffusion method. It was found that the causes behind antibacterial activity of *L. coryniformis* subsp. *torquens* MTi1 are acids and H_2O_2 , as it lost its antibacterial activity after adjusting to neutral pH and treated with catalase. On the other hand, *L. coryniformis* MTi2 retained its antibacterial activity against *E. coli* (15mm) and *B. subtilis* (14mm) even after adjusting to neutral pH and treated with catalase. This implicate that this isolate could produce bacteriocin or bacteriocin like substances (Table 2)¹⁸.

Searching new or better candidate of probiotic is focused and continuously progressing research interest. Based on empirical data gathered

from in vitro experiments in this study, we can conclude that isolated *L. coryniformis* subsp. *torquens* MTi1 and *L. coryniformis* MTi2 have great potentiality to be used as probiotics after passing through other relevant in vitro and in vivo experiments.

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