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GROWTH INHIBITION OF *SALMONELLA* BY *LEUCONOSTOC* SPECIES ISOLATED FROM BUFFALO MILK CURD

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Abstract

Traditional fermented products are considered to be a niche for microbial diversity. Probiotics offer a unique approach for addressing the threat of antibiotic resistance. Therefore, the aim of the present study was to isolate *Leuconostoc* spp. from Buffalo milk curd, an artisanal fermented dairy product in Bangladesh and assess their growth inhibition competence against *Salmonella* sp. A total of 50 isolates were isolated and purified from five freshly prepared buffalo milk curd samples using two different media, with glucose or sucrose as the carbon source. Among these pure isolates, 37 were identified as presumptive *Leuconostoc* spp. The biochemical identification using the VITEK 2 system confirmed that 59.5% of the isolates were *Leuconostoc mesenteroides* ssp. *cremoris*, while 40.5% were *Leuconostoc pseudomesenteroides*. Out of the identified isolates, twelve had a similarity of over 97%, and twenty-five had a similarity of less than 96%. Consequently, these twelve isolates were tested for their antimicrobial activity against *Salmonella* sp. using two *in vitro* methods, agar well diffusion and microbroth. The isolate M1L1 exhibited the highest inhibitory zone (15 mm) and showed 55.6% growth inhibition by the agar well diffusion and microbroth assay, respectively, suggesting a similar efficacy between the two methods. Notably, five other isolates also displayed inhibitory zones ranging from 13 to 15 mm and growth inhibition percentages of 37 to 52%. Overall, the isolated *Leuconostoc* spp. could be utilized as probiotics to combat pathogenic microorganisms.

Keywords: VITEK 2, antimicrobial, agar well diffusion assay, microbroth assay.

Introduction

Dahi, often referred to as curd*,* is considered to be one of the oldest fermented milk products throughout the Indian sub-continent and often considered as an analogue to the western fermented product called 'yogurt'. Curd or *dahi* is extensively prepared in Bangladesh using both cow milk and buffalo milk. Buffalo milk curd is one of the naturally fermented traditional dairy products found in *Bhola* district, the largest island in southern-central Bangladesh. The product is fermented by the native microflora of raw buffalo milk that gets entrance to the milk from the environment. Moreover, the milk used to prepare this traditional dairy product comes from those dairy buffaloes often grazing on the coastal grasslands. Therefore, the possibility of

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microbial diversity in these products is very high. According to the scientific reports (Landry *et al*., 2017), traditional dairy products especially those made from raw milk are rich in numerous both useful and harmful microbiota. The useful microorganisms are generally non-starter lactic acid bacteria (NSLAB). However, the origin and quality of milk, environmental conditions like livestock diet (grass, hay etc.), seasons, altitude of pasture, and manufacturing process of dairy products, and hygienic conditions during manipulation of the milk all have a significant impact on what microbial populations are present (Tilocca *et al*., 2020).

The species from the genera *Leuconostoc* are heterofermentative lactic acid bacteria used as components of mesophilic cultures to produce aroma during milk fermentation. Presently, the genus *Leuconostoc* has 13 species that metabolize the citrate of milk to produce diacetyl (Hemme and Focaud-Scheunemann, 2004), a key odor compound in butter, buttermilk, and certain cheese varieties (Smit *et al*., 2005). They also participate in the formation of other aroma and flavor compounds, such as lactic, acetic acid, and ethanol (Hemme and Focaud-Scheunemann, 2004). These and other metabolic end products contribute not only to the flavor profile of fermented products but, via their antimicrobial action, to their preservation (Hemme and Focaud-Scheunemann, 2004). The antimicrobial activity of *Leuconostoc* species has been demonstrated by several research findings e.g. *Leuconostoc* against *Listeria monocytogenes*, *Listeria innocua*, *Lactobacillus plantarum*, *Listeria ivanovii*, *Staphylococcus aureus* (Fatma and Benmechernene, 2013); *E. coli*, *Staphylococcus aureus*, *Salmonella enteritidis* (Wang *et al*., 2018). Some dairy *Leuconostoc* strains are reported to produce pediocin-like bacteriocins (Sawa *et al*., 2010), which may further contribute to food safety while reducing food spoilage. Interestingly, the genus includes good antagonist activity, although it has also been linked to deteriorating food activities.

Though their numbers may vary widely, *Leuconostoc* species have been reported to be present in many traditional dairy products made from raw milk with or without starters (Nieto-Arribas *et al*., 2010). Together with the mesophilic lactobacilli, *Leuconostoc* species contribute to the NSLAB populations of dairy environments. As NSLAB members, they are deemed to play a pivotal role in maintaining flavor and typicity of traditional dairy products (Nieto-Arribas *et al*., 2010). These products therefore provide a reservoir of phenotypic and genetic biodiversity, from which new strains with novel properties might be selected for improving adjunct cultures.

Probiotics are living microorganisms that not only improve the balance of intestinal microflora but also inhibit many pathogens (FAO/WHO, 2011). They are widely used in animal production because of their antibacterial activity and various biological characteristics. Probiotics are labeled as GRAS (Generally Recognized as Safe). As the curd made in *Bhola* is made solely from raw milk without any pasteurization, there could be a risk of pathogen contamination, especially species from *Salmonella.* No research has ever been conducted to study the effect of *Leuconostoc* spp. from the native microflora on pathogens, especially *Salmonella* species, in curd made from buffalo milk available in *Bhola* district. Therefore, this study was designed

to isolate *Leuconostoc* species from naturally fermented traditional Buffalo milk curd and test their antimicrobial competence against *Salmonella* species.

Materials and Methods

Sample collection

Five freshly prepared buffalo milk curd samples were collected from five different manufacturers located in the *Bhola* district. Since buffalo milk comes from different chars (small islands), the manufacturers were selected based on the source of milk. Buffalo milk curd was mixed properly onsite using a sterilized spatula and around 50 g was collected in pre-sterilized polystyrene tubes. The samples were transported to the laboratory of the Dairy and Poultry Science Department, BSMRAU maintaining cold chain by using ice box.

Enumeration and isolation of presumptive *Leuconostoc* spp.

Samples were analyzed for enumerating *Leuconostoc* spp. on modified plate count agar supplemented with 0.5 g/L Tween 80, 5.0 g/L ammonium citrate, 1 g/L skim milk powder, 0.04 g/L $FeSO_4$, 0.2 g/L $MgSO_4$, 0.05 g/L $MnSO_4$ and 10.0 g/L glucose (Media-1), and sucrose-enriched agar containing 10 g tryptone/liter, 5 g yeast extract/liter, 10 g sucrose/liter, 20 g $CaCO_3$/liter, 15 g agar/liter (Media-2). In the present study, two types of media were used to observe the differences of carbon sources in enumerating and isolating *Leuconostoc* spp. Glucose was sterile-filtered separately and added after autoclaving. Both media were supplemented with vancomycin for selective isolation of *Leuconostoc*. The plates were incubated at 22°C for 5 days before colony enumeration (Alegría *et al*., 2012; Frantzen *et al*., 2017). After incubation, well-separated single colonies having different morphologies from each dilution of different plates were randomly selected for isolation and identification.

Biochemical identification

The phenotypic identification of all the isolates was performed by the VITEK-2 compact analyzer (bioMérieux, France) which followed the determination of the biochemical profile as well. The bacterial suspension was standardized (0.5 McFarland) using 0.85% saline solution. The test suspension tube was then placed into the cassette with a GP-2 reagent card (for gram-positive cocci and non-spore-forming bacilli) and loaded into the machine for incubation in accordance with the number of samples. As illustrated in Table 1, after performing 43 biochemical tests, the final identification results were available in 8 hours.

Determination of antimicrobial activity

The antimicrobial activity of the isolated *Leuconostoc* spp. against *Salmonella* sp. was performed by two methods: the agar well diffusion assay (Fatma and Benmechernene, 2013) and microbroth method (Arena *et al*., 2016). The *Salmonella* sp. used in this study was previously isolated from the chicken intestine using selective media (Salmonella Shigella Agar, HiMedia Pvt. Ltd. India). The black-coloured colony with negative Gram staining properties identified the isolates as presumptive *Salmonella* spp. The pure colonies were preserved at -86°C with 30% glycerol in the laboratory of the Dairy and Poultry Science Department, BSMRAU.

Table 1. Test parameters for the biochemical identification using the VITEK 2 system

Sl. No.	Test ID	Abbreviated test parameter
1	AMY	D-Amygdalin
2	PIPLC	Phosphatidylinositol phospholipase C
3	dXYL	D-Xylose
4	ADH1	Arginine dihydrolase 1
5	BGAL	Beta galactosidase
6	AGLU	Alpha-glucosidase
7	APPA	Ala-Phe-Pro Arylamidase
8	CDEX	Cyclodextrine
9	AspA	L-Aspartate Arylamidase
10	BGAR	Beta Galactopyranosidase resorufine
11	AMAN	Alpha-mannosidase
12	PHOS	Phosphatase
13	LeuA	Leucine Arylamidase
14	ProA	L-Proline Arylamidase
15	BGURr	Beta - glucoronidase
16	AGAL	Alpha-galactosidase
17	PyrA	L-Pyrrolydnyl - Arylamidase
18	BGUR	Beta-glucoronidase
19	AlaA	Alanine Arylamidase
20	TyrA	Tyrosine Arylamidase
21	dSOR	D-Sorbitol
22	URE	Urease
23	POLYB	Polymyxin-B resistance
24	dGAL	D-galactose
25	dRIB	D-ribose
26	ILATK	L-Lactate alkalinisation
27	LAC	Lactose
28	NAG	N-Acetyl-D-glucosamine
29	dMAL	D-Maltose
30	BACI	Bacitacin resistance
31	NOVO	Novobiocin resistance
32	NC6.5	Growth in 6.5% NaCl
33	dMAN	D-Mannitol
34	dMNE	D-Mannose
35	MBdG	Methyl-B-D-Glucopyranoside
36	PUL	Pullulane
37	dRAF	D-Raffinose
38	0129R	O/129 Resistance (comp.vibrio)
39	SAL	Salicin
40	SAC	Saccharose/Sucrose
41	dTRE	D-Trehalose
42	ADH2S	Argininine Dihydrolase 2 (Thioglycolate sigma)
43	OPTO	Optochin Resistance

The agar well diffusion assay

The cell-free culture supernatant of each *Leuconostoc* isolate was obtained by removing cells from overnight grown MRS cultured broth (inoculum 1%, v/v, 22°C for 24h) through centrifugation (10,000 rpm for 10 min at 4°C). The supernatant was then adjusted to pH 6.5 by 1M NaOH and filter sterilized (0.22 μm). After preparing, the surface of the Mueller-Hinton Agar plates was punched using the backside of a sterile 1 ml micro-tip equivalent to 6 mm diameter. The overnight grown *Salmonella* sp. in Luria-Bertani broth were washed and re-suspended in fresh Luria-Bertani broth equivalent to an optical density of 0.5 at 600 nm (cell suspension) and then inoculated onto the Mueller-Hinton Agar plates by swab technique. The cell-free culture supernatant (80 μl) was transferred into each 6 mm hole and incubated at 37°C for 24h. After incubation, the inhibitory zones were measured by examination of the diameters of any clear zones around the wells using a caliper (Wang *et al*., 2018).

Bacteriostatic effect of cell-free supernatant using micro broth method

The target *Salmonella* sp. grown overnight in Luria-Bertani broth was inoculated into fresh Luria-Bertani broth to achieve an optical density of 0.5 at 600 nm. The cell-free culture supernatant of each *Leuconostoc* isolate was prepared as mentioned earlier. A 200 μl volume of test solution, consisting of 100 μl of the cell suspension and 100 μl of cell-free supernatant was seeded in a 96-well microplate followed by mixing. The plate was then incubated at 37°C for 24h. The growth was monitored by measuring optical density at 600 nm using a microplate reader. The growth inhibition percent was calculated using the following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{Absorbance of the culltured media without supernatant-Absorbance the cultured media with supernatant}}{\text{Absorbance of the culltured media without supernatant}} \times 100$$

Data interpretation

The collected data were recorded, organized in a Microsoft Excel worksheet. The analyses were done using the Statistical Package for the Social Sciences (SPSS) software (SPSS 20). The data were expressed as mean±SD. One-way ANOVA was carried out to evaluate the level of significance. The means were compared using Duncan's multiple range test (DMRT) and $p<0.05$ were considered as significant.

Results and Discussion

Enumeration and isolation of presumptive *Leuconostoc* spp.

The viable colonies of presumptive *Leuconostoc* spp. were enumerated after spreading the Buffalo milk curd samples on two different types of media. As shown in Fig. 1, the log CFU/g of sample in Media-1 and Media-2 were 8.1±1.1 and 6.8±0.9, respectively. The viable cell count in Media-2 was found lower than that of Media-1. This could be due to the differences in carbon

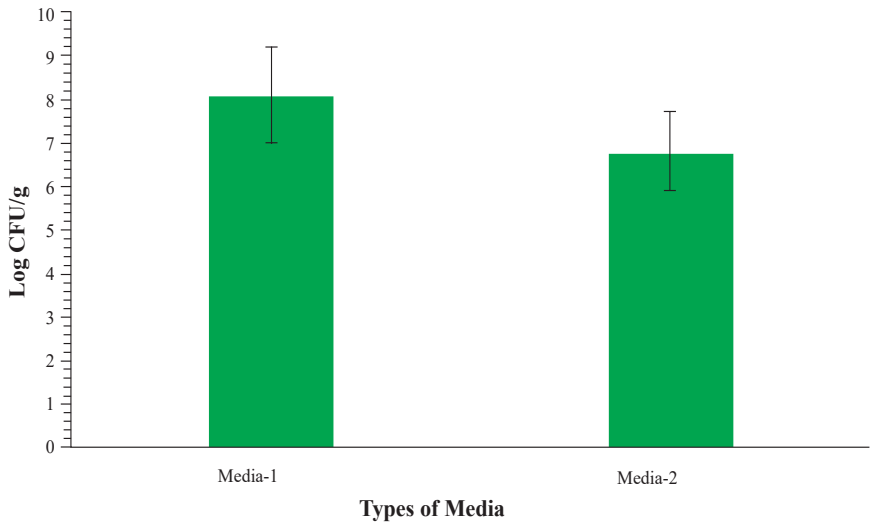

Fig. 1. Enumeration of viable colonies of presumptive *Leuconostoc* on two different media.

sources of two media. Sucrose-enriched media (Media-2) was reported to enumerate the dextran producing *Leuconostoc* (Alegría *et al*., 2012) while the modified agar media containing glucose as carbon source was revealed to isolate different species of *Leuconostoc* (Frantzen *et al*., 2017).

From the two *Leuconostoc* selective agar media (Media-1 and Media-2), a total of 50 colonies (25 from each media) were randomly selected. The isolates were marked as M1L1 to M1L25 for Media-1 and M2L1 to M2L25 for media-2. M1 and M2 stand for Media -1 and Media-2, respectively, while L and the number adjacent to the letter indicate *Leuconostoc* and isolates number, respectively. Of the 50 colonies, 37 appeared as small in size, greyish in color and slimy. The representative image of the purified colonies shown in Fig. 2 (a) and (b) revealed the colonies. The morphological characteristics in Gram staining test confirmed the isolates as cocci shaped with chain and gram-positive as shown in Fig. 2 (c). The isolates were also found as catalase negative and non-motile. Thus, 16 isolates from Media-1 and 21 isolates from Media-2 were finally identified as presumptive pure *Leuconostoc* spp. Members of the *Leuconostoc* genus are reported to be catalase-negative, gram-positive cocci usually arranged in pairs or chains and often found in plants, dairy products, and foods (Arias and Murray, 2015).

Biochemical identification of presumptive *Leuconostoc* spp.

Based on the results of VITEK 2 system using VITEK 2 GP card, it was found that all the isolates belonged to either *Leuconostoc mesenteroides* ssp. *cremoris* or *Leuconostoc pseudomesenteroides*. Both species were able to grow on Media-1 and Media-2 (Table 2) suggesting no differences in carbon source during isolation. Of the 37 isolates, 22 (59.5%) were identified as *Leuconostoc mesenteroides* ssp. *cremoris* and 15 (40.5%) were recognized as *Leuconostoc pseudomesenteroides* (Table 2). All the isolates were tested for 43 different

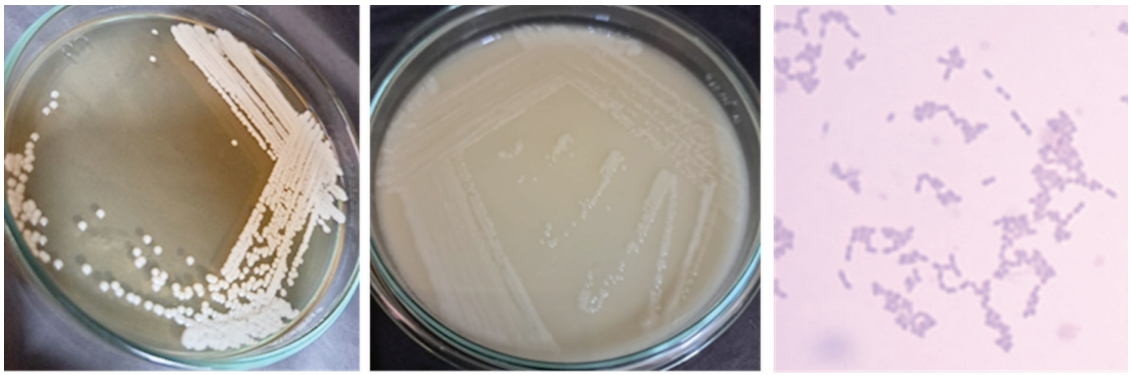

Fig. 2. Representative images of purified colonies grown in Media-1 (a) and Media-2 (b), and Gram staining of pure isolate (c), respectively.

test parameters as mentioned in Table 1. The VITEK 2 system identified the isolates according to the test results as present in Table 2. Twelve isolates displayed >97% similarities and the remaining 25 isolates showed <96% similarities (Table 2). Of these 12 isolates 6 were identified as *Leuconostoc mesenteroides* ssp. *Cremoris* and another 6 were confirmed as *Leuconostoc pseudomesenteroides*.

Antimicrobial activity against *Salmonella* sp. using agar well diffusion assay

As presented in Table 3, all the tested isolates showed low to moderate inhibition against *Salmonella* sp. The diameter of the inhibitory zone ranged from 08 to 15 mm. The highest inhibitory zone was observed in the isolate M1L1, M1L13 and M2L17 (zone diameter 15 mm) followed by M1L8 and M2L7 (zone diameter 14 mm) and M2L2 (zone diameter 13 mm). The lowest inhibitory zone was detected by M1L4 (zone diameter 08 mm) followed by M2L9 and M2L11 (zone diameter 09 and 10 mm, respectively). The representative image of inhibition zone against *Salmonella* sp. by agar well diffusion method has been presented in Fig. 3. A recent study revealed that *Leuconostoc pseudomesenteroides* showed inhibition activity against *Salmonella enteritidis* in the well diffusion assay with an inhibitory zone diameter of 11 to 22 mm (Wang *et al*., 2018).

Antimicrobial activity against *Salmonella* sp. using microbroth assay

The microbroth method was applied to observe the antimicrobial activity of the 12 isolates against *Salmonella* sp. As shown in Fig. 4, the growth of *Salmonella* sp. was inhibited in an isolate dependent fashion. The growth inhibition ranged from 18.2 to 55.6%. The highest inhibition was observed with M1L1 (55.6%) followed by M2L17 (52.1%) and the lowest inhibition was revealed by M1L4 (18.2%), M2L9 (19.4%) and M2L4 (22.7%). The growth inhibition ability of the isolate M1L1 differs significantly ($p<0.05$) in all the isolates except M2L17. In contrary, there were no significant differences among M1L4,

Table 2. Biochemical assessment of *Leuconostoc* spp. by VITEK 2 system and suggesting organisms

Isolate ID	Organism identified	% Similarity
M1L1	*Leuconostoc pseudomesenteroides*	99%
M1L3	*Leuconostoc pseudomesenteroides*	99%
M1L4	*Leuconostoc pseudomesenteroides*	98%
M1L7	*Leuconostoc pseudomesenteroides*	92%
M1L8	*Leuconostoc mesenteroides* ssp. *cremoris*	99%
M1L9	*Leuconostoc mesenteroides* ssp. *cremoris*	91%
M1L11	*Leuconostoc pseudomesenteroides*	92%
M1L18	*Leuconostoc mesenteroides* ssp. *cremoris*	93%
M1L19	*Leuconostoc mesenteroides* ssp. *cremoris*	50%
M1L21	*Leuconostoc mesenteroides* ssp. *cremoris*	50%
M1L12	*Leuconostoc pseudomesenteroides*	92%
M1L13	*Leuconostoc mesenteroides* ssp. *cremoris*	98%
M1L14	*Leuconostoc pseudomesenteroides*	96%
M1L15	*Leuconostoc mesenteroides* ssp. *cremoris*	99%
M1L22	*Leuconostoc mesenteroides* ssp. *cremoris*	51%
M1L24	*Leuconostoc mesenteroides* ssp. *cremoris*	50%
M2L1	*Leuconostoc mesenteroides* ssp. *cremoris*	89%
M2L12	*Leuconostoc pseudomesenteroides*	92%
M2L13	*Leuconostoc mesenteroides* ssp. *cremoris*	50%
M2L16	*Leuconostoc pseudomesenteroides*	92%
M2L19	*Leuconostoc mesenteroides* ssp. *cremoris*	91%
M2L20	*Leuconostoc mesenteroides* ssp. *cremoris*	90%
M2L23	*Leuconostoc mesenteroides* ssp. *cremoris*	50%
M2L2	*Leuconostoc mesenteroides* ssp. *cremoris*	99%
M2L3	*Leuconostoc mesenteroides* ssp. *cremoris*	94%
M2L4	*Leuconostoc mesenteroides* ssp. *cremoris*	99%
M2L5	*Leuconostoc pseudomesenteroides*	92%
M2L7	*Leuconostoc pseudomesenteroides*	99%
M2L24	*Leuconostoc mesenteroides* ssp. *cremoris*	91%
M2L9	*Leuconostoc pseudomesenteroides*	99%
M2L11	*Leuconostoc mesenteroides* ssp. *cremoris*	98%
M2L14	*Leuconostoc pseudomesenteroides*	96%
M2L6	*Leuconostoc mesenteroides* ssp. *cremoris*	95%
M2L17	*Leuconostoc pseudomesenteroides*	98%
M2L21	*Leuconostoc pseudomesenteroides*	92%
M2L22	*Leuconostoc mesenteroides* ssp. *cremoris*	50%
M2L25	*Leuconostoc mesenteroides* ssp. *cremoris*	89%

Table 3. Antimicrobial activity against *Salmonella* sp. using agar well diffusion assay

Sl. No.	Isolates name	Zone diameter (mm)
1	M1L1	15.2±0.3
2	M1L3	12.1±0.1
3	M1L4	08.4±0.1
4	M1L8	14.3±0.4
5	M1L13	15.3±0.1
6	M1L15	11.1±0.2
7	M2L2	13.2±0.4
8	M2L4	11.2±0.4
9	M2L7	14.4±0.2
10	M2L9	09.1±0.3
11	M2L11	10.3±0.2
12	M2L17	15.4±0.4

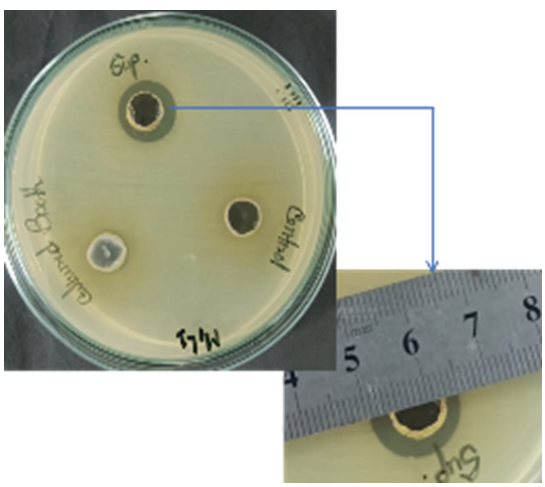

Fig. 3. Representative image of inhibition zone against *Salmonella* sp. by agar well diffusion method.

M2L4 and M2L9. Again, M1L15 and M2L2, M1L3 and M2L7, M1L13 and M2L17, M1L8 and M2L2, M2L4, M2L9 and M2L11 were not significantly different in terms of growth inhibition ability. Interestingly, the results of the microbroth method and well diffusion method was highly correlated.

Discussion

Traditional native food products like buffalo milk curd made from raw milk can serve as valuable resources for future food production. In this study, to explore the diversity of *Leuconostoc* spp., we isolated 50 *Leuconostoc* spp. isolates obtained from five traditional buffalo milk curd samples from five different producers and biochemical identification confirmed 37 isolates as *Leuconostoc* spp.

Several lactic acid bacteria (LAB) have been found to combat *Salmonella* sp. LAB are known for their production of various antimicrobial substances such as organic acids, hydrogen peroxide, bacteriocins, antibiotic compounds which contribute to their ability to inhibit the growth of pathogenic microorganisms (Vieco-Saiz *et al*., 2019). Organic acids produced from LAB have been considered inhibitory metabolites that repress pathogen growth (Neal-McKinney *et al*., 2012). According to Holzapfel (2002), There is indeed an increasing need to select

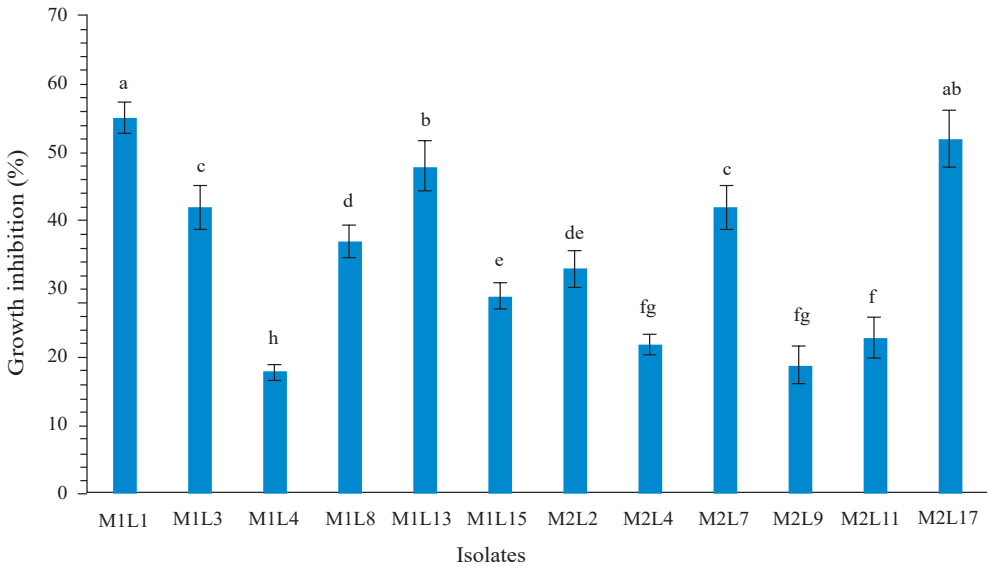

Fig. 4. Antimicrobial activity against *Salmonella* sp. by microbroth method. The values are the mean±SD; bar with different small letter (a, b, c, d, e, f, g or h) differs significantly ($p<0.05$).

microbial strains with specific functional properties for commercial production and to improve the quality and safety of traditional fermented food products. *Leuconostoc* spp. are commonly used as adjunct cultures in food production, particularly in combination with fast acid-producing *Lactococcus* spp. These mixed type starter cultures contribute to the aroma and texture formation of the final food products through various metabolic activities through citrate degradation and production of diacetyl, acetoin, and carbon dioxide (Özcan *et al*., 2019). Wang *et al*., 2018 reported *Leuconostoc pseudomesenteroides* showed inhibition activity against *Escherichia coli* (*E. coli* ATCC 25922), *Staphylococcus aureus* and *Salmonella enteritidis* in the well diffusion assay and showed the inhibitory effect against *S. enteritidis* by displaying a zone diameter of 15 mm which corresponds to the present study. *L. mesenteroides* and *Leuconostoc* spp. are reported to produce antimicrobial compound namely leucocin (Benmechernene *et al*., 2013; Yusuf and Hamid, 2013). In the present antimicrobial assay performed using the isolates, we observed a clear inhibition zone of approximately 15 mm caused by *Leuconostoc* spp. The supernatant of the isolates used for the antimicrobial assay was adjusted to pH 4.0, indicating that *Salmonella* sp. was not inhibited by organic acids such as lactic acid or other acids. Therefore, the antimicrobial effect of *Leuconostoc* spp. might result from bacteriocins; however, information regarding bacteriocins produced by *Leuconostoc* spp. is limited. So, additional studies investigating this bactericidal effect are required.

Conclusion

The present study showed moderate *in vitro* antimicrobial efficacy against *Salmonella* sp. by five *Leuconostoc* isolates. As the

curd made from raw buffalo milk by native microflora without any heat treatment could pose a health risk, inhibition of pathogens like the *Salmonella* sp. by the native microflora is promising advancement. It thus makes sense regarding safety of buffalo milk curd so well regarded in *Bhola*. Since the antimicrobial activity is considered as one of the important probiotic characteristics, it is logical to explore other *in vitro* probiotic properties and also *in vivo* study. The genetic analysis of these isolates is also equally important. The true inhibiting factor from *Leuconostoc* spp. should also be detected. Therefore, further study is needed towards developing future probiotic candidate for animals and humans.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

Alegría, A., P. Szczesny, B. Mayo, J. Bardowski and M. Kowalczyk. 2012. Biodiversity in Oscypek, a traditional Polish cheese, determined by culture-dependent and -independent approaches. *Appl. Environ. Microbiol*. 78(6): 1890-8. doi: 10.1128/AEM.06081-11.

Arena, M. P., A. Silvain, G. Normanno, F. Grieco, D. Drider, G. Spano and D. Fiocco. 2016. Use of *Lactobacillus plantarum* strains as a bio-control strategy against food-borne pathogenic microorganisms. *Front. Microbiol*. 7: 464. https://doi: 10.3389/fmicb.2016.00464.

Arias, C. A and B. E. Murray. 2015. *Enterococcus* species, *Streptococcus gallolyticus* group, and *Leuconostoc* species. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. https://doi.org/10.1016/b978-1-4557-4801-3.00202-2.

Benmechernene, Z., H. F. Chentouf, B. Yahia, G. Fatima, M. Quintela-Baluja, P. Calo-Mata and J. Barros-Velázquez. 2013. Technological aptitude and applications of *Leuconostoc mesenteroides* bioactive strains isolated from Algerian raw camel milk. *Biomed Res. Int*. 2013: 418132.

Fatma, C. H and Z. Benmechernene. 2013. Isolation and identification of *Leuconostoc mesenteroides* producing bacteriocin isolated from Algerian raw camel milk. *Afr. J. Microb. Res*. 7(23): 2961-2969.

Food Agriculture Organization/World Health Organization [FAO/WHO]. 2011. Expert Consultation on the Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. Cordoba: FAO.

Frantzen, C. A., W. Kot, T. B. Pedersen, Y. M. Ardö, J. R. Broadbent, H. Neve and H. Holo. 2017. Genomic characterization of dairy associated *Leuconostoc* species and diversity of Leuconostocs in undefined mixed mesophilic starter cultures. *Front. microb.* 8: 132.

Hemme, D and C. Foucaud-Scheunemann. 2004. *Leuconostoc*, characteristics, use in dairy technology and prospects in functional foods. *Int. Dairy J*. 14(6): 467-494.

Holzapfel, W. H. 2002. Appropriate starter culture technologies for small-scale fermentation in developing countries. *Int. J. Food Microb*. 75(3): 197- 212.

Landry, B. K. U., Z. N. François, R. Y. Wang, Z. Taicheng and Y. Li. 2018. Viability and stress response of putative probiotic *Lactobacillus plantarum* strains in honey environment. *Probiotics and antimicrobial proteins*. 10(4): 629-637. https://doi.org/10.1007/s12602-017-9358-7.

Mayeaux, J., W. Sandine and P. Elleker. 1962. A selective medium for detecting *Leuconostoc* organisms in mixed-strain starter culture. *J. Dairy Sci*. 45(5): 655.

Neal-McKinney, J. M., X. Lu , T. Duong, C. L. Larson, D. R. Call, D. H. Shah and M. E. Konkel. 2012. Production of organic acids by probiotic lactobacilli can be used to reduce pathogen load in poultry. *PLoS ONE*. 7(9). e43928. https://doi.org/10.1371/journal.pone.0043928.

Nieto-Arribas, P., S. Seseña, J. M. Poveda, L. Palop and L. Cabezas. 2010. Genotypic and technological characterization of *Leuconostoc* isolates to be used as adjunct starters in Manchego cheese manufacture. *Food Microb*. 27(1): 85-93.

Ozcan, E., S. S. Selvi, E. Nikerel, B. Teusink, E. T. Oner, T. Çakır. 2019. A genome-scale metabolic network of the aroma bacterium *Leuconostoc mesenteroides* subsp. cremoris. *Appl. Microbiol. Biotech*. 103: 3153-3165.

Sawa, N., K. Okamura, T. Zendo, K. Himeno, J. Nakayama and K. Sonomoto. 2010. Identification and characterization of novel multiple bacteriocins produced by *Leuconostoc pseudomesenteroides* QU 15. *J. appl. microb*. 109(1): 282-291. https://doi.org/10.1111/j.1365-2672.2009.04653.x.

Tilocca, B., N. Costanzo, V. M. Morittu, A. A. Spina, A. Soggiu, D. Britti and C. Piras. 2020. Milk microbiota: Characterization methods and role in cheese production. *J. proteomics*. 210: 103534. https://doi.org/10.1016/j.jprot.2019.103534.

Vieco-Saiz, N., Y. Belguesmia, R. Raspoet, E. Auclair, F. Gancel, I. Kempf and D. Drider. 2019. Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Front. microb*. 10: 57.

Wang, Y., A. Li, X. Jiang, H. Zhang, K. Mehmood, L. Zhang and J. Li. 2018. Probiotic potential of *Leuconostoc pseudomesenteroides* and *Lactobacillus* strains isolated from yaks. *Front. microb*. 9: 2987. https://doi.org/10.3389/fmicb.2018.02987.

Yusuf, M. A and T. H. Hamid. 2013. Lactic acid bacteria: bacteriocin producer: a mini review. *IOSR J. Pharm.* 3(4): 44-50.