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Isolation and Analysis of Probiotic Properties of *Lactobacillus spp*. from Selective Traditional Curd (Sangom Aphamba)

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Abstract: *Traditional curd (Sangom Aphamba) is considered as a potential source of probiotics. In the present study, Lactobacillus spp. were isolated from five traditional curd samples collected from Khangabok named as (LS1K), Haorang Keirel (LS2HK), Oinam (LS3O), Mayang Imphal (LS4MI) and Sanjenbam (LS5SJ) regions in Manipur and one sample of curd prepared from boiled milk (LS6LMS) for the isolation of Lactobacillus spp. Single colonies were isolated by serial dilution method on MRS (de Man, Ragosa, and Sharpe) agar plate by subsequent streaking. Isolated strains were analysed for probiotic properties including acid (pH) and NaCl tolerance, sugar fermentation and catalase test. Acid tolerance test was performed in MRS broth with pH 2, 4 ,6 & 8 and NaCl tolerance in MRS broth with 2%, 4%, 6% and 8% conc. It was observed that all the isolates except LS3O(Oinam) were able to survive in highly acidic pH 2 and survived in 2% and 4% NaCl concentrations. Sugar fermentation tests showed that the isolates were able to ferment the test sugar. This study indicated that the Lactobacillus spp. isolated from traditional curd (Sangom Aphamba) samples have potential probiotic properties.*

Key words: *Lactobacillus spp., traditional curd, Sangom Aphamba, probiotic.*

1. INTRODUCTION:

Sangom Aphamba, a popular fermented milk product in Manipur which is made in an earthen pot (previously fired at high temperature) is consumed in many households for its beneficial properties. Within the group of lactic acid bacteria, *Lactobacillus* species are the most commonly utilized group of microorganisms for their potential beneficiary as probiotics (Morrow et al., 2012). The major factor which is considered before selecting probiotic strain is the survival of probiotic bacteria in gastrointestinal tract which includes tolerance under acidic conditions, bile salts, low pH, NaCl and its antimicrobial properties against pathogenic bacteria (Petros et al., 2006; Durme et al., 2001; Issazadeh et al., 2013). Therefore, the present study was undertaken with the following objectives:

- Isolation and identification of *Lactobacillus spp*. from Sangom Aphamba of different regions in Manipur.
- Sugar fermentation of the isolated lactobacilli.
- Determination of acid and NaCl tolerance.

2. MATERIALS AND METHODS

Sample collection: Sangom aphamba samples from Khangabok, Haorang Keirel, Oinam, Mayang Imphal and Sanjenbam regions were collected in sterile container and stored at 4℃. Nomenclature of the isolates were carried out representing the initials of my Name, Sample number (numeric figure) and place of the sample. For example, LS1K indicates Luxmida Sample number 1 Khangabok.

Isolation of the bacteria: 1ml of each sangom aphamba samples were serially diluted from 10^{-1} to 10^{-5}, 10^{-1} to 10^{-6}, 10^{-1} to 10^{-7} respectively for each sample. Spread plate techniques were performed from 10^{-3} to 10^{-7} tubes for the entire sample by using MRS agar (selective media for *Lactobacillus spp.*) and incubated anaerobically at 37℃ for 24-48 hrs. Isolated colonies were selected from 10^{-4}, $10^{-5}$and 10^{-6} dilutions. After subsequent re-streaking of the colonies in MRS medium isolated pure colonies were grown and stored at 4℃ for further analysis.

Catalase Test: With the help of inoculation loop a small amount of colony was transferred in a clean surface of dry glass slide. A drop of 3% H_2O_2 solution was mixed with the culture.

Acid tolerance test: For determination of acid tolerance test, fresh overnight bacterial cultures were inoculated in 10ml MRS broth at pH 2, 4, 6 and 8. The pH was adjusted with 10N HCl and 1N NaOH. The inoculated broths were incubated for 24 hrs at 37℃.

NaCl tolerance test: For determination of NaCl tolerance test, fresh overnight bacterial culture inoculated in 10ml MRS broth with different NaCl concentrations of 2, 4, 6 and 8% of NaCl. After 24 hrs incubation bacterial growth was determined by observing their turbidity.

Sugar fermentation test: Sugar fermentation test was performed using 1% (w/v) sugar in Nutrient broth for all the isolates. Glucose, sucrose, mannitol and starch were used in this test. Phenol red solution was used as an indicator. 5ml media was dispensed in a test tube and Durham's tube was inverted in each of the test tube and sterilized at 121℃ for 15 minutes. Fresh cultures were inoculated and incubated at 37℃ for 24 hr. Sterile media was used as a negative control.

3. RESULTS:

All the isolates were catalase negative (no air bubbles were observed). The sugar fermentation results were observed by colour changing from red to acidic yellow and gas formation in durham's tube (Goyal et al., 2010). The isolates from Khangabok (LS1K) region were able to ferment all the test sugar (glucose, sucrose, mannitol and starch) with no gas formation whereas isolates from Haorang Keirel (LS2HK), Mayang Imphal (LS4MI) and Sanjenbam (LS5SJ) regions were not able to ferment starch. The isolates from Oinam region (LS3O) were not able to ferment all the sugars. However, the isolates from Laboratory made sample (LS6LMS) were able to ferment all the test sugars. All the isolates except LS3O (Oinam) have the ability to tolerate acid as low as pH 2 and showed growth in MRS broth containing different NaCl concentration, however it showed less growth in 8% NaCl conc.

Table 1: Biochemical characterization of the isolated lactobacilli from Sangom aphamba

		Isolate1 LS1K	Isolate2 LS2HK	Isolate3 LS3O	Isolate4 LS4MI	Isolate5 LS5SJ	Isolate6 LS6LMS
Catase Test		-VE	-VE	-VE	-VE	-VE	-VE
Sugar fermentation	Glucose	+VE	+VE	+VE	+VE	+VE	+VE
	Sucrose	+VE	+VE	+VE	+VE	+VE	+VE
	Mannitol	+VE	+VE	+VE	+VE	+VE	+VE
	Starch	+VE	-VE	-VE	-VE	-VE	+VE

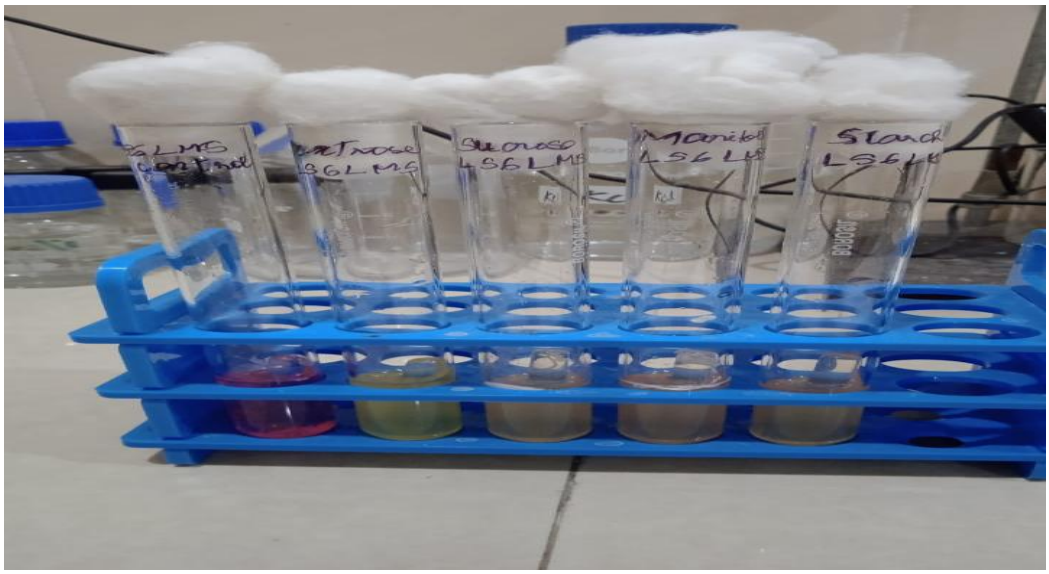

Fig 1: Fermentation of the test sugar by the isolates (colour changes from red to acidic yellow).

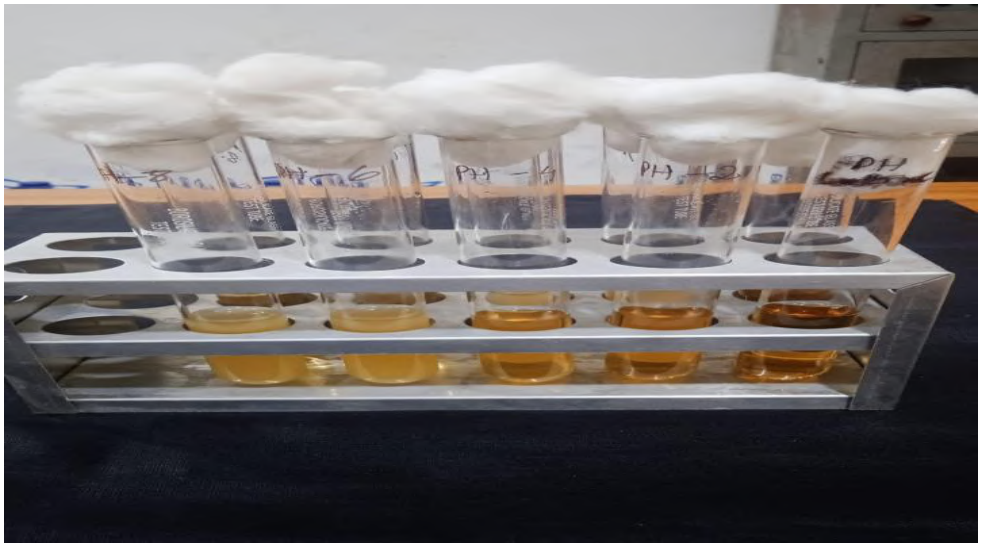

Fig 2(a): Tolerance to pH (turbidity in the media shows the growth of bacteria)

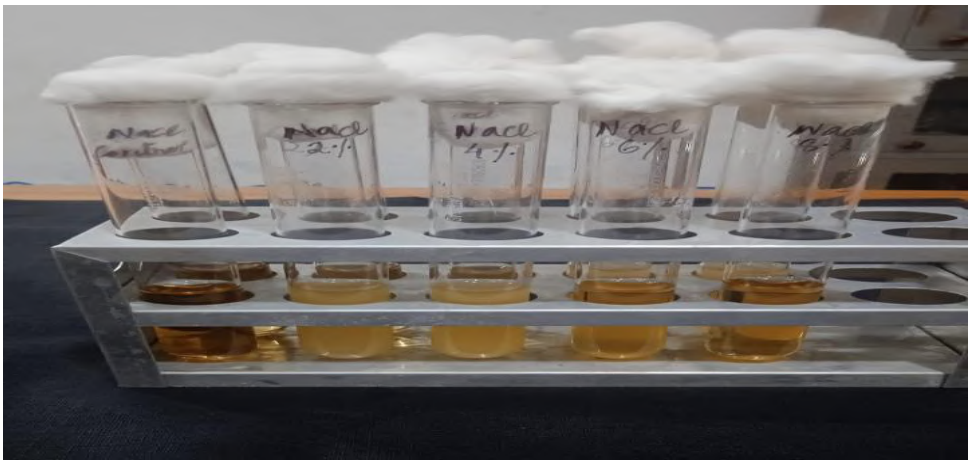

Fig 2(b): Tolerance to NaCl conc. (turbidity in the media shows the growth of bacteria)

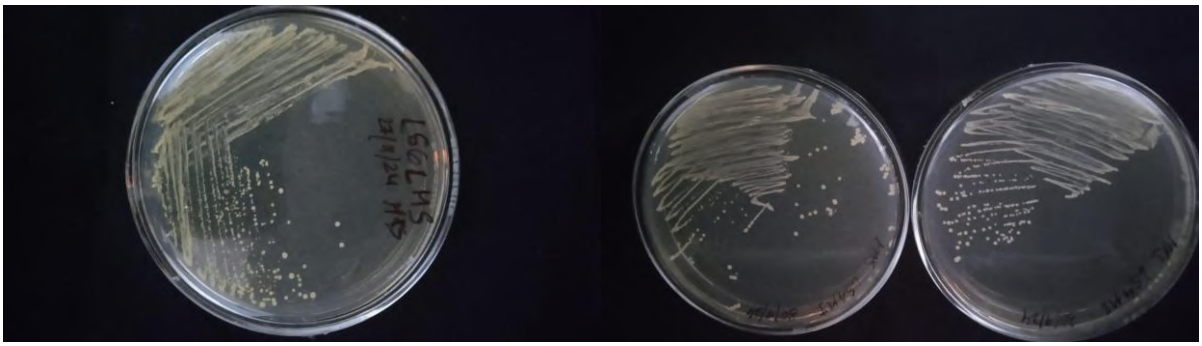

Fig 3: Isolation of *Lactobacillus* sps from different Sangom aphamba samples

Table 2: Tolerance to acid and NaCl of isolated *Lactobacilli* from Sangom aphamb

Strain	pH Tolerance				NaCl Tolerance			
	2	4	6	8	2%	4%	6%	8%
Isolate 1(LS1K)	+	++	+++	+++	+++	+++	++	+
Isolate2 (LS2HK)	+	+	+++	++	+++	+++	++	+
Isolate 3 (LS3O)	-	-	-	-	-	-	-	-
Isolate 4 (LS4MI)	+	+	+++	++	+++	+++	+	+
Isolate 5(LS5SJ)	+	+	+++	++	+++	+++	++	+
Isolate 6 (LS6LMS)	+	+	+++	+++	+++	+++	++	+

+= slight growth; ++= moderate growth; +++= dense growth

4. DISCUSSION:

The present study was undertaken to find the *Lactobacillus* spp from Sangom aphamba, a traditional curd in Manipur. In this experiment, five different *Lactobacillus* spp isolated from 5 different region Khangabok (LS1K), Haorang Keirel (LH2HK), Oinam (LS3O), Mayang Imphal (LS4MI), Sanjenbam (LS5SJ) and one laboratory made sample from Boiled milk. The isolated *Lactobacilli* were catalase negative and able to ferment test sugar (glucose, sucrose, mannitol, starch) without producing gas while the isolates LS2HK, LS4MI,LS5SJ did not able to ferment starch (table 1, fig: 1). In this study, survivability in different pH was performed for all strains in both acidic (as low as Ph 2) and alkaline condition (pH 8). It was observed that isolated *Lactobacilli* showed slight growth at acidic pH 2 and dense growth at alkaline pH 8 (table 2, fig: 2). Tolerance to NaCl is also one of the probiotic characteristics of *Lactobacillus* spp. In this study, the isolates showed maximum growth in 2% and 4% NaCl conc. and less growth in 6% and 8% conc.of NaCl. These findings are similar to the findings of Soni et al; 2021.

5. CONCLUSION:

In the present study, *Lactobacillus* spp. were isolated from six different sangom aphamba samples and evaluated for their probiotic properties. All isolates except LS3O, demonstrated the ability to survive under acidic and alkaline conditions, as well as in high concentrations of NaCl. These findings indicate that the majority of the isolates possess promising probiotic potential. Further studies are needed to assess the isolates' antimicrobial activity against pathogenic bacteria, resistance to bile salts and digestive enzymes. In vitro studies and genomic analyses would also be valuable to

confirm the safety and efficacy of these strains as potential probiotic candidates for functional food or therapeutic applications.

REFERENCES

1. Morrow L.E., Gogineni V., and Malesker M.A, (2012): Probiotics in the intensive care unit. *Nutr. Clin. Prac*. *27,* 235-241.
2. Petros A.M., Georgia Z., Christos M., George K., Bruno P., and Effie T, (2006): Probiotic potential of *Lctobacillus* strains isolated from dairy products. *Int. Dairy J. 16*: 189-199. http://doi.org/10.1016/j.idairyj.2005.02.009
3. Durme C., Mahony L., Murphy L., Thornton G., Morrissey D., and Hallorari S, (2001): *In Vitro* selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. *Am. J. Clin. Nutr. 73*:386-392. https://doi.org/10.1093/ajcn/73.2.386s
4. Issazadeh K., Ali Abdali M.A., Darsanaki R.K., Alikhani F., Dadras H., and Tajehmiri A, (2013): Identification and Analysis of probiotic properties of *Lactobacillus spp.* From Traditional Yoghurts in North of Iran. *J. Pure Appl. Microbiol*. *7*(4):2965-2971.
5. Goyal R., and Dhingra H, (2010): Isolation and identification of genus *Lactobacillus* from different curd samples. *Biosciences, Biotechnology Research Asia. 7*(2), 907-912.
6. Soni M., Shah H.R., and Patel S.M, (2021): Isolation, Identification and Analysis of Probiotic Characteristics of Lactobacillus spp. from Regional Yoghurts from Surendranagar District, Gujarat. *Asian Journal of Dairy and Food Research*. Vol issue: DR-1631 (1-6).