Antimicrobial Activity of *Lactobacillus* spp. Isolated from Traditional Curd (Sangom Aphamba) Against Selected Pathogens

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ABSTRACT

Traditional Manipuri curd (Sangom aphamba) is considered a rich source of probiotics. The genus *Lactobacillus* comprises a large group of beneficial bacteria that produce lactic acid as the end product of fermentation. The present study focuses on the isolation and analysis of the the antimicrobial activity of *Lactobacillus* spp. from traditional curd samples.

A total of five curd samples were collected from Khangabok (LS1K), Haorang Keirel (LS2HK), Oinam (LS3O), Mayang Imphal (LS4MI) and Sanjenbam (LS5SJ), along with one laboratory prepared curd sample (LS6LMS). Isolation was carried out using MRS (de Man, Ragosa, and Sharpe) agar, followed by purification through repeated streaking. Based on the morphological and biochemical characteristics of the isolates, six strains of *Lactobacillus* spp. were identified.

The isolates were evaluated for antagonistic activity against *Micrococcus luteus* (MTCC 106), *Bacillus subtilis* (MTCC 121) and *Escherichia coli* (MTCC 739). The isolates exhibited notable antimicrobial activity against *M. luteus* and *E. coli*, while no inhibition was observed against *B. subtilis*. Among the isolates, LS5SJ demonstrated the highest zone of inhibition (14.5mm) against *E. coli*, followed by LS2HK (13.5 mm) and LS1K (11 mm).

The findings suggest that traditional curd (Sangom aphamba) contains *Lactobacillus* spp. with significant antagonistic properties, supporting its role as a beneficial food.

Key words: *Lactobacillus*, curd, antimicrobial activity, antagonistic, probiotic.

INTRODUCTION

Traditional curd (Sangom aphamba) is obtained by Lactic acid fermentation of milk. Due to the increasing health concern among the population, there has been rapid increase in the consumption of fermented milk product as it is believed to have probiotics. Consumption of curd has been reported to cure diarrhoea (Agarwal and Bhasin, 2002); and reported to have anti-diabetic (Yadav et al., 2007); anticarcinogenic (Arvind *et al*., 2010) properties.

Lactic acid bacteria (LAB) are broadly classified into genera such as *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Bifidobacterium* and *Pediococcus* (Pathak and Dutta, 2016).

The *Lactobacilli* comprises a large and diverse group of Gram-positive non-sporulating, non-motile rods or cocci that are associated to produce lactic acid as the major end product of carbohydrate fermentation (Pelinesco *et al*., 2009). Various strains of *Lactobacilli* provide additional health benefits such as bile tolerance (Walker and Gilliland, 1983), gastric juice tolerance (Kilara, 1982) and antibiotic resistance (Curragh and Collins, 1992). Some of the beneficial effects of curd are attributed to the antibacterial components formed during the fermentation and the low pH that prevents the growth of putrefactive and other undesirable organisms including potential pathogens and possesses an increased digestibility (Conly *et al*., 2004).

Gut microbiota plays an essential role in digestion, metabolism and immune function. Changes in the diversity and function of this ecosystem has been associated with bowel disorders, inflammatory immune diseases, insulin resistance and infectious diseases caused by *Clostridium difficile*. Dysbiosis as a result of antibiotics usage or the presence of different pathogenic organisms can be prevented or reduced by probiotic consumption (Arqués et al., 2015).

Probiotics, or the live organism which when administered in an adequate amount, confer a health benefit on the host in the control of intestinal pathogens. Antimicrobial activity is considered as a probiotic trait. Most probiotics applied in food products are lactic acid bacteria, mainly *Lactobacillus and Bifidobacterium*. Therefore, the present study was carried out to evaluate the antimicrobial activity of traditional curd (Sangom aphamba) with respect to the presence of antagonistic *Lactobacillus spp.*

MATERIALS AND METHODS

i) **Collection of Samples:** Sangom aphamba samples from Khangabok, Haorang Keirel, Oinam, Mayang Imphal and Sanjenbam regions of Manipur were collected in a sterile container and stored at 4℃. A laboratory-prepared curd sample (LS6LMS) was also included. Nomenclature of the isolates were carried out by representing the initials of my name, sample number (numeric figure) and place of the sample. For example, LS1K indicates Luxmida Sample number1 Khangabok and one laboratory made curd sample from boiled milk named as LS6LMS.

ii) **Isolation and Maintenance of *Lactobacillus:*** Isolated strains from different sangom aphamba samples was done on de Man, Ragosa, Sharpe (MRS) agar medium. Single colonies were obtained by subsequent streaking on MRS medium and incubated anaerobically at 37℃ for 24-48 hrs. Isolated pure colonies were grown and stored at 4℃ for further analysis.

iii) **Identification of *Lactobacillus:*** *Lactobacillus* strains isolated in the study were identified on the basis of morphological and biochemical tests according to Bergey's manual of systematic bacteriology (Holt et al., 1994, Williams and Wilkins, 1994).

   a) **Morphological Characteristics:** Morphological examination of the isolates were done by Gram staining method for identification and confirmed to be Gram positive bacteria (Fig- 1,2).

   b) **Biochemical Characteristics:** Catalase test and Sugar fermentation were done for the biochemical characteristics of the isolates. Glucose, sucrose, mannitol and starch were used as a test sugar (Table 1).

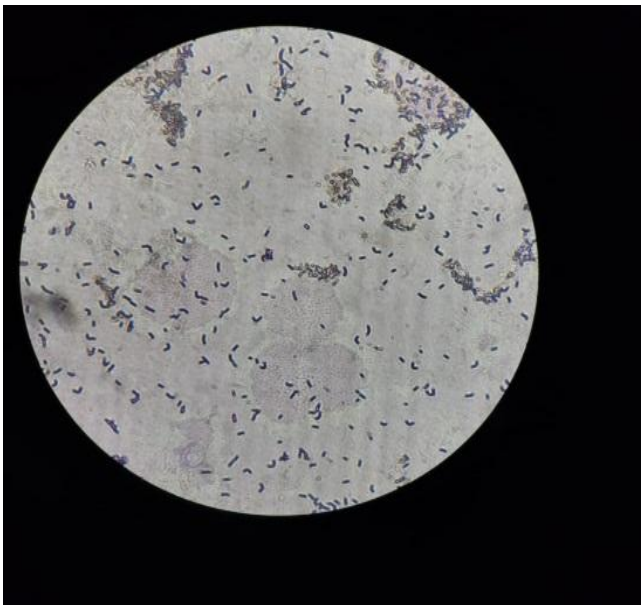

Fig1: Gram staining image of *Lactobacillus* sp. in 100X

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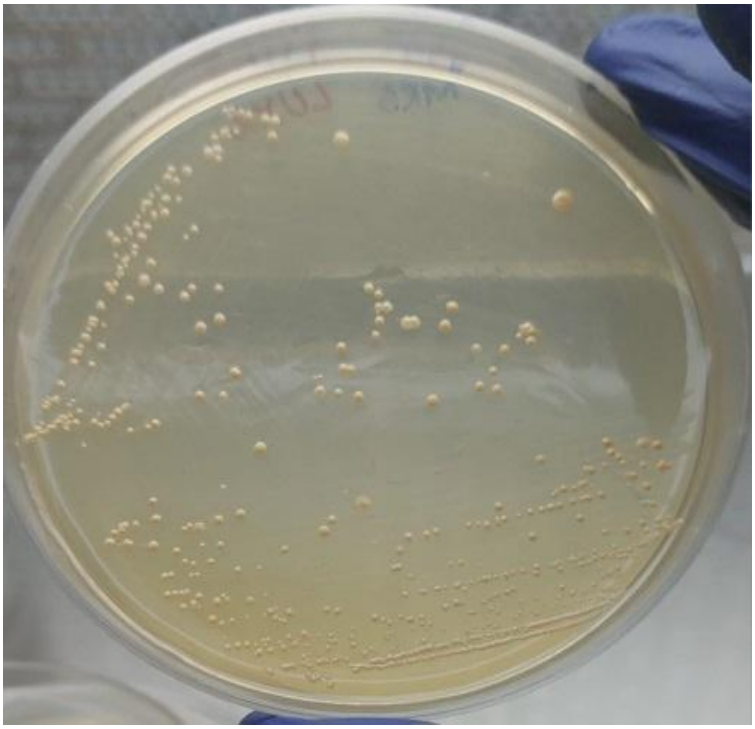

Fig 2: Isolation of *Lactobacillus* sp. from Sangom aphamba

Table 1: Biochemical Characterization of *Lactobacilli* from Sangom Aphamba

		Isolate1 (LS1K)	Isolate2 (LS2HK)	Isolate3 (LS3HK)	Isolate4 (LS4MI)	Isolate5 (LS5SJ)	Isolate6 (LS6LMS)
Catalase 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

Antagonistic Activity of *Lactobacillus* Against Selected Bacterial Pathogens:

Antimicrobial activity was assessed using the agar well diffusion method (Tagg and McGiven, 1971). Test pathogens (*M. luteus*, *B. subtilis*, and *E. coli*) were cultured on nutrient agar plates. Wells were filled with 0.1 ml of *Lactobacillus* supernatant and incubated at 37°C for 24–48 hours. One plate of N.A was kept as control. Zones of inhibition were measured in millimetres (Fig-3).

RESULTS

The isolated *Lactobacillus* strains demonstrated varying levels of antagonistic activity:

- **LS1K:** 11 mm inhibition against *M. luteus* (MTCC 106) and *E. coli* (MTCC 739); none against *B. subtilis* (MTCC 121)
- **LS2HK:** 13.5 mm inhibition against *E. coli* (MTCC 739); none against others
- **LS3O & LS4MI:** No inhibition observed

- **LS5SJ:** Highest activity with 14.5 mm against *E. coli* (MTCC 739) and 12 mm against *M. luteus* (MTCC 106)
- **LS6LMS:** No activity

Overall, *E. coli* was the most susceptible organism, followed by *M. luteus*, while *B. subtilis* showed complete resistance.

The antagonistic activities of isolated *Lactobacillus* strains isolated from traditional curd, Sangom aphamba were screened against 3 pathogenic bacteria obtained from Microbial type culture collection viz. *Micrococcus luteus (MTCC 106), Bacillus subtilis (MTCC 121) and Escherichia coli (MTCC 739)*. Among these *Lactobacillus* strains, LS1K showed 11mm inhibition zone against *Micrococcus luteus (MTCC 106)* and 11mm against *Escherichia coli (MTCC 739),* 0.0mm against *Bacillus subtilis (MTCC 121).* LS2HK showed 13.5mm inhibition zone against *Escherichia coli (MTCC 739)*, 0.0 mm against *Micrococcus luteus (MTCC 106*) and *Bacillus subtilis (MTCC 121*). LS3O and LS4MI does not showed any zone of inhibition against the tested pathogen. LS5SJ observed the highest zone of inhibition among the 6 lactobacillus strains with diameter 12mm against *Micrococcus luteus (MTCC 106),* 0.0mm against *Bacillus subtilis (MTCC 121),* and 14.5mm against *Escherichia coli (MTCC 739).* LS6LMS showed 0.0mm against all the tested pathogen. Among the tested pathogens*, E. coli* was the most inhibited strain followed by *Micrococcus luteus*. None of the 6 isolated strains were antagonistic against *Bacillus subtilis*. In this study, LS5SJ was found to be most antagonistic strain, Fig 4(i). However, LS1K was found to be least antagonistic strain, Fig 4(iii).

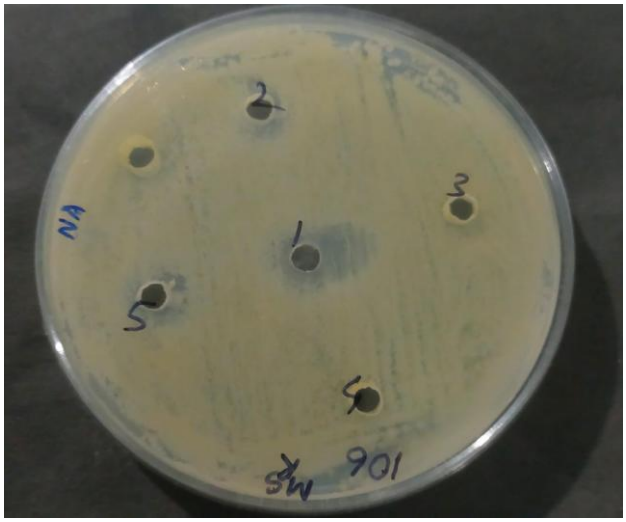

Fig 3: Antagonistic activity of *Lactobacillus* sp. from Sangom aphamba against MTCC 106 (*M. luteus*)

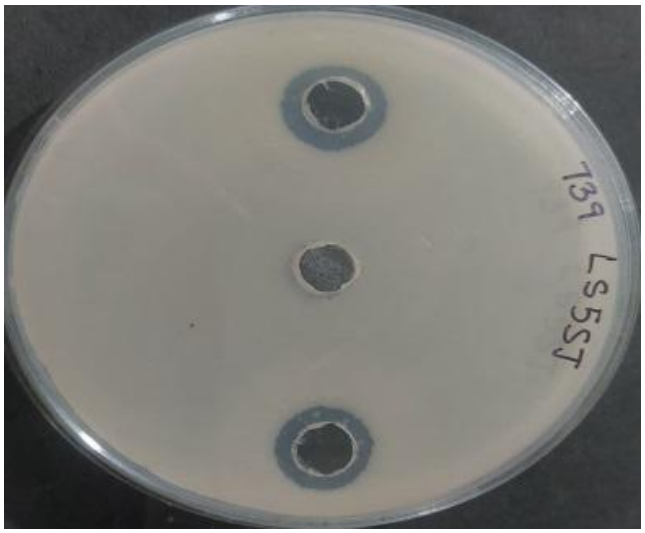

Fig 4(i)- LS5SJ showed the highest zone of inhibition against *E. coli* with 14.5mm diameter



Fig 4(ii)- LS2HK against *E. coli* with diameter 13.5mm

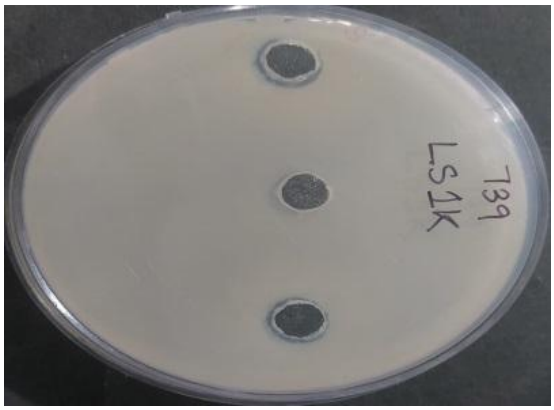

Fig 4(iii)- LS1K showed 11.5mm diameter against *E. coli.*

CONCLUSION

The study demonstrates that traditional curd (Sangom aphamba) contains *Lactobacillus* spp. with notable antimicrobial activity. Among the isolates, LS5SJ showed the strongest antagonistic effect. These findings support the probiotic potential of traditional curd and its role in promoting health through inhibition of pathogenic bacteria.

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