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Effect of selected Lactic acid bacteria based-starter cultures on development of curds and their antibacterial properties against some enteric pathogens

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Abstract
As many as 77 lactic acid bacterial strains were isolated from various sources. Out of which 10 promising isolated were based on various probiotic and functional traits. They were further subjected for antibacterial activity tested against common enteric human pathogens such as *Listeria monocytogenes, Salmonella typhimurium, Escherichia coli and Staphylococcus aureus.* Firstly, the pathogens were tested for their growth on de Man Rogosa Sharpe medium over which these isolates were investigated for their antibacterial activity and zone of inhibition was recorded. Thus, these promising 10 isolated showed a good antibacterial activity which further can be utilized for the development of curds using UASD, Dairy milk. They were also checked with curds prepared with standard industrial cultures. Hence, these isolates hold potential to be used as Lactic acid bacterial starter cultures for the development of curds.

Keywords: Lactic acid bacteria, isolates, curds, antibacterial activity.

Introduction
Lactic acid bacteria (LAB) are Gram positive, non-spore forming, Catalase negative, acid tolerant, Fastidious, non-motile and facultative anaerobic friendly gut bacteria. They constitute heterogeneous group of industrially important bacteria. In food industries, they used as preservative, acidulant and flavoring agents by the virtue of their probiotic traits. They are also in use as starter culture in food fermentations such as beverages, yoghurt, vegetables, cereals, meat, cocoa beans etc. (Doyle and Beauchat, 2007) [6]. They are also utilized in the development of functional foods and more specifically their application as vaccines, pro and prebiotics, Neutraceuticals has attracted new research arena for food scientists and health professionals (Mozzi *et al.,* 2010; De Vuyst *et al*., 2004) [7, 5].
Traditionally, the fermented foods serve as good sources of new potential sources of LAB. Therefore use of strains of such origin would be of great importance, consumers who suffer from lactose intolerance. In order to exert its beneficial effect on the host, a probiotic strain must be able to survive the gut passage of humans to reach to the action site in viable state and should be in sufficient population (normally 10^7 cells per ml). Thus, in order to survive in and colonize the gut, the bacteria should express high antibacterial activity. They are supplemented as probiotics that are live microbial food supplements which beneficially affect the host by improving the intestinal microbial balance. Hence, as many as 25 isolates of lactic acid bacteria were obtained from various sources such as traditional artisan curds, fermented vegetables, dosa and idli batter, etc. The present work was aimed to evaluate the probiotic potential of these LAB isolates in terms of acid and bile tolerance properties.

Materials and Methods
Isolation of LAB strains, media and culture conditions
As many as 25 LAB strains were isolated from various sources such as traditional fermented arisan curds of Karnataka such as Kohlar, Banashankari, Almatti areas, fermented vegetables etc. by the method of plate count method. The 48 h grown culture was further purified and maintained on de Mann Rogosa Sharpe broth (MRS) (De Man *et al.,* 1960) at -80^0 C by DMSO (Cry protectant) method.

Test for antibacterial properties
The isolates will be tested for their antibacterial activity against common human pathogens (*Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*) (Arasu *et al*., 2013) [3].

Media for growth of pathogens

The pure cultures of food borne pathogens namely *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* were inoculated from slants to brain heart infusion broth (BHIB). After 24hr in incubation at 37°C, the culture broth was centrifuged and the pellet obtained was suspended in 9ml Saline. This suspension was used for inoculation of the pathogenic strain to Nutrient agar plates for the antimicrobial activity determination of the sample filtrate.

Antimicrobial activity

To check the antimicrobial activity, the MRS agar plates be overlaid with 7 ml of soft MRS agar inoculated with 20 µl of overnight active culture of indicator strains. Make different wells in agar and fill wells with 50 µl cell free broth of 24 h old cultures obtained by centrifuging the culture broth at 5000 rpm for 15 min. The broth be neutralized to pH 6.5 and also inoculated into wells. The diameter of zone of inhibition extending laterally around the well be measured and a clear zone of 1 mm or more to be considered positive inhibition.

Inhibition of *E. Coli* and *Salmonella* spp. by lactic acid bacteria were examined using the method of Ahn *et al.* (1997). MRS broth containing lactic acid bacteria (10^2 CFU/ml) were incubated with the same number of *E. Coli* or *Salmonella* spp, cells at 37 and 42°C for isolates

The isolates were tested for their antibacterial activity against human pathogens (*Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysentriae*, *Salmonella typhimurium*) (Arasu *et al.*, 2013) [3].

Aly Savadogo *et al.* (2003) [2] Isolated eighty strains of lactic acid bacteria producing bacteriocin were isolated from Burkina Faso fermented milk samples. These strains were identified to species *Lactobacillus fermentum*, *Pediococcus* sp., *Leuconostoc mesenteroides* and *Lactococcus.* Isolated bacteriocin exhibited antibacterial activity against *Enterococcus faecalis*, *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* using the agar drop diffusion test. The inhibition diameters obtained with bacteriocin are between 8mm and 12mm. Gram positive indicator bacteria were most inhibited.

Results and discussion

When antibacterial activity of the LAB isolates were tested for *in vitro* antagonistic activity against *Listeria monocytogenes* it was interesting to note that as many as 42 isolates showed inhibition against *Listeria monocytogenes*. The highest zone of inhibition of 23.5 mm dia. was exhibited by isolate no.3 followed by isolate no. 72 (21mm dia.). Similarly, 68 isolates exhibited inhibition against *Salmonella typhimurium*. The highest activity was observed by strain no. 75 (23.5 mm dia.) followed by isolate no 40 and 58 with 22.5 mm dia. each. 58 isolates exhibited inhibitory effect against *E. coli* (Table 1).

Table 1: Anti-bacterial activity of LAB isolated against common human enteric pathogens measured in mm (Zone of inhibition).

LAB Isolates	*Listiria monocytogenes*	*Salmonella typhimurium*	*Eschrichia coli*	*Staphylococcal aureus*
1	0.00	15.50	22.50	0.55
2	21.00	17.50	20.50	0.00
3	23.50	15.50	0.00	0.00
4	0.00	18.00	15.50	0.00
5	16.50	17.00	18.00	0.00
6	18.00	12.50	20.50	0.00
7	18.00	16.50	18.00	0.00
8	17.00	16.50	17.00	0.00
9	14.00	15.50	18.00	0.00
10	13.50	0.00	0.00	14.00
11	18.00	15.50	22.00	11.00
12	17.50	16.00	18.50	0.00
13	16.00	15.50	17.50	0.00
14	16.00	17.50	17.50	0.00
15	0.00	15.50	22.50	13.00
16	8.50	14.00	24.00	11.50
17	19.00	0.00	21.50	0.00
18	9.50	14.50	21.00	0.00
19	0.00	0.00	15.50	0.00
20	0.00	12.00	0.00	0.00
21	0.00	12.50	17.00	0.00
22	14.00	15.50	17.00	0.00
23	15.00	17.00	14.50	0.00
24	0.00	16.50	15.50	0.00
25	0.00	14.50	17.00	0.00
26	0.00	20.50	18.00	0.00
27	0.00	15.50	15.50	0.00
28	0.00	0.00	0.00	12.50
29	0.00	0.00	0.00	0.00
30	0.00	15.00	14.50	10.50
31	0.00	15.00	17.00	0.00
32	0.00	14.00	21.50	0.00
33	12.00	19.50	15.50	13.00
34	0.00	21.50	0.00	0.00
35	12.50	14.50	13.50	0.00
36	0.00	15.50	0.00	0.00
37	16.50	17.50	16.50	0.00

38	14.00	16.50	20.50	0.00
39	10.00	19.00	17.00	0.00
40	20.00	22.50	18.50	0.00
41	0.00	15.00	0.00	0.00
42	17.00	19.00	18.50	0.00
43	0.00	20.50	17.50	0.00
44	0.00	15.00	0.00	0.00
45	13.00	18.50	21.50	0.00
46	19.00	16.50	0.00	0.00
47	13.50	19.00	12.50	10.00
48	18.00	20.50	23.00	12.50
49	17.00	16.50	20.00	0.00
50	0.00	0.00	20.00	0.00
51	0.00	18.50	17.50	0.00
52	0.00	19.50	21.00	0.00
53	0.00	17.50	22.50	0.00
54	0.00	21.00	20.50	6.00
55	0.00	20.50	20.50	0.00
56	8.00	16.50	23.50	0.00
57	0.00	17.50	0.00	0.00
58	15.00	22.50	23.50	0.00
59	17.00	16.50	0.00	0.00
60	0.00	0.00	16.00	0.00
61	0.00	0.00	15.50	10.50
62	17.50	14.50	0.00	0.00
63	0.00	15.50	0.00	0.00
64	0.00	0.00	10.50	12.50
65	0.00	16.00	0.00	0.00
66	0.00	18.00	17.50	0.00
67	0.00	17.50	18.50	0.00
68	0.00	19.50	20.50	0.00
69	0.00	16.00	14.50	10.00
70	18.00	19.50	21.50	0.00
71	0.00	17.50	0.00	0.00
72	21.00	21.50	12.50	5.50
73	0.00	20.50	22.00	0.00
74	0.00	20.00	0.00	10.00
75	0.00	23.50	13.50	9.50
76	0.00	16.50	0.00	0.00
77	0.00	20.50	0.00	0.00
SEM ±	1.76	1.03	1.47	0.31
CD @ 0.01%	6.57	3.87	5.49	1.14

The highest anti *E. coli* activity was exhibited by isolate no.16 (Zone of inhibition, 24mm dia.) followed by isolate numbers 56 and 58 (23.5 mm dia. each). Only 14 isolates showed inhibitory effect against *Staphyloccus aureus*. Isolate no. 10 showed the highest zone of inhibition of 14 mm dia. followed by isolate no. 33 with 13 mm dia.

In another study conducted by reported that the addition of cinnamon or licorice did not change yogurt fermentation but sustain the growth of *lactobacillus ssp.* during refrigerated storage. Cinnamon-yogurt or licorice-yogurt containing probiotic bacteria inhibited the growth of *H. pylori* in vitro. The effectiveness of these herbal –yogurt to halt the growth of *H. pylori* needs to be further investigated under extremely acidic environment of the stomach.

A study by rozila *et al.*, (2012) [8] showed that samples of fresh raw goat's milk and goat's yogurt were collected from two different farms in Selangor and Kedha respectively. Twenty three isolates of LAB were obtained and thirteen were successful identified as LAB based on classical identification through morphological, biochemical and gram stained. Four isolates were positive as Lactococci and nine isolates were assigned as lactobacillus genera. Then, these isolates were proceeded to screen the antibacterial activity which conducted using spot-on-lawn and disc diffusion methods. All isolates were observed to behave antagonistic against all indicator strains with differences in size of inhibition zone ranging between 13 mm to 25mm in diameter. From the result obtained, *Listeria monocytogenes, Enterococcus faecalis, Enterococcus faecium* and *Enterobacter aerogenes* were being inhibited by these LAB isolates meanwhile *E. coli* 0157 and *Pseudomonas aeruginosa* were not inhibited except isolates SUA10 and ARB6 respectively.

reports indicate, the diameters of inhibition zones were varied it ranged between 0.6 to 4 mm. this revealed that the LAB inhibited all the pathogenic bacteria and inhibition was scored positive if the width of the clear zone around the colonies of the producer strain was 0.5 mm or larger. Similar study was carried out in Morocco by Kalalou whose studied the activity of LAB on some gram positive and negative pathogenic bacteria such as *E. coli, Pseudomonas aeroginosa*, *Klebsiella pneumonia, Staphylococcus aureus* and *Bacillus cereus* and the inhibition zone were in the range of 1.4 to 2.8 cm.

Conclusion

In conclusion, among the 77 LAB isolates used in the study showed most of the screened lactic acid bacteria showed antagonistic effect against commonly occurring human enteric pathogens. These isolates have a great potential and prospective candidates as one of the probiotic traits to carry forward for further study in the preparation of yoghurt. Thus

this will be carry out to evaluate comprehensively on the mechanism of the antagonism properties displayed.

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