

## Potency Antibacterial Properties of Probiotic Acido-Bifido-Yogurt Against *E. coli*

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### ABSTRACT

Probiotic Acido-bifido-yoghurt was prepared by using buffalo milk standardized to 18% TS. The routine yoghurt starter cultures (i.e. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*) along with a proven strain of probiotic *Lactobacillus acidophilus* and a human strain of *Bifidobacterium bifidum* were inoculated in the suitably heat processed milk (90°C/15 min.). The Cell-free culture (CFC) filtrates were obtained from the inoculated milk at an interval of 6, 12, 18, 24 and 30 h. Antibacterial activity of CFC from normal yoghurt without any other cultures, *Acidophilus* milk, *Bifidus* milk and probiotic Acido-bifido-yoghurt was studied using cup-well assay against mastitic strain of *Escherichia coli*. It was further observed that the antibacterial effect of the CFC of the products under investigation was highest at 24 h followed by which there was a gradual decline in the effect. It was concluded from this study that when these four culture organisms are grown in association, they exert a synergistic antibacterial effect on the test organisms.

**Key words:** *Acidophilus* milk, *Bifidus* milk, LAB, *E. coli*

### INTRODUCTION

In recent years, numerous studies have been published on the health effects of yogurt and the bacterial cultures used in the production of yogurt. In the United States, these lactic acid-producing bacteria (LAB) include *Lactobacillus* and *Streptococcus* species the benefits of yogurt and LAB on gastrointestinal health have been investigated in animal models and, occasionally, in human subjects<sup>11</sup>. Yoghurt is a coagulated milk product obtained by lactic acid fermentation by *Streptococcus salivarius* ssp. *thermophilus*

and *Lactobacillus delbrueckii* ssp. *bulgaricus*. It is an established fact that the enormously increasing popularity of yoghurt is primarily based on the biochemical performance of lactic cultures. One of the most important properties of the LAB is their antagonistic properties. Griffiths *et al.*<sup>6</sup>, reported inhibition of two Gram-negative bacteria in raw milk stored at low temperature by two commercial preparations of mesophilic LAB. The LAB concentration was 10<sup>8</sup> cfu/ml and the pH decrease was in the order of 1 unit.

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Richardson<sup>13</sup> observed that the growth of *Pseudomonas fluorescens* in sterile milk at 7°C was practically stopped by inoculation of 2X10<sup>8</sup> cfu/ml of *Leuconostoc mesenteroides* subsp. dextranicum LL223 with a pH decrease of 1.3 units. Ross<sup>14</sup> studied the antibiosis capacity of different strains against spoilage microorganisms in milk and attributed to inhibitory principles produced by LAB. Moreover, the use of raw milk to study the effect of LAB has the advantage of producing results in accordance with industrial practice<sup>4</sup>. Alm<sup>1</sup> reported that a daily consumption of more than 500 ml acidophilus milk shortened the duration of Salmonella carrier state in randomly selected human carriers.

Prasad and Gandhi<sup>12</sup> studied some factors affecting the production of antibacterial substances in *L. acidophilus* strain-R against fourteen different types of microorganisms. *Micrococcus flavus* was the most sensitive organism to antibacterial activity of *L. acidophilus* strain-R and the highest activity was reported in sterilized skim milk as compared to cow's whole milk, buffalo milk, goat milk and a whey-lactose-yeast extract medium. Studies of Attaie *et al*<sup>2</sup>., indicated that inhibition of Staph. aureus was highest during acidophilus-yoghurt production as compared to standard yoghurt and acidified yoghurt production. Danielson *et al*<sup>5</sup>., reported higher antimicrobial activity of *L. acidophilus* against *B. subtilis* than *E. coli* when grown in MRS broth supplemented with 0.2 or 0.4 % bile salts. The aforementioned antimicrobial effect is attributed to antibiotic/antibiotic-like products, acid(s), hydrogen peroxide and some unidentified/partially identified compounds by the LAB<sup>8</sup>. The probiotic acidobifido yoghurt extend more health benefit over normal yoghurt<sup>3</sup> Like psychrotrophs, coliforms can also reduce the diacetyl content of buttermilk and sour subsequently producing a yogurt-like flavor. In cheese production, slow lactic acid production by starter cultures favors the growth and production of gas by coliform bacteria, as coliforms having short generation times under such conditions. In soft, mold-ripened cheeses, the pH increases during

ripening, which increases the growth potential of coliform bacteria<sup>9</sup>.

The most common food-borne pathogens of major concern, to public health, in ready-to-eat foods are coliform bacteria as general indicator for fecal pollution, and in particular *Escherichia coli* O157:H7, *Salmonella* spp, *Listeria monocytogenes* and *Bacillus cereus* as well as the presence of coagulase-positive *Staphylococcus aureus* and their ability to produce various types of their enterotoxin<sup>10</sup>. The present investigation was planned and executed to ascertain the antimicrobial characteristics of Acido-bifido-yoghurt against mastitic strain of *Escherichia coli*.

## MATERIAL AND METHODS

**Method for preparation of probiotic Acido-bifido-yoghurt:** Raw buffalo milk was procured and standardized to milk fat content of 5% and 10% SNF by using fresh skim milk. The culture organisms were activated in fresh, unsupplemented buffalo skim milk. The Acido-bifido-yoghurt was prepared by inoculating the drastically thermal processed standardized milk with 1% active culture of *Bif. bifidum* and incubating the bulk container for one hour followed by addition of 1% inoculum of a human strain of *L. acidophilus*-LBKV<sub>3</sub> and incubation for one more hour. It was followed by 1% normal yoghurt culture (1:1) and incubated further at 40° c till desire acidity. The product thus obtained was analyzed for various parameters like titratable acidity, viable counts, sensory attributes along with anti-microbial properties

**Source of the organisms:** The test cultures of pathogenic organism namely, *E. coli* used in this investigation were obtained from the National Chemical Laboratory (under Council of cultures Scientific & Industrial Research, Govt. of India, New Delhi), Pune. The pure culture of a proven probiotic strain of *L. acidophilus*-LBKV<sub>3</sub>, a human vaginal isolate was obtained from its original source<sup>8</sup>. While the pure yoghurt culture and that of *Bif. bifidum*-NCDC 255 was obtained from National Collection of Dairy Cultures,

Division of Dairy Microbiology, National Dairy Research Institute, Karnal (Haryana).

**Assay for antimicrobial properties:** The method described by Ibrahim and Bezkorovainy<sup>7</sup> was followed with slight modifications. In order to know the antibacterial activity of the various cultures individually and in combinations against selected pathogenic organisms, the cell-free-culture (CFC) filtrate was used. To prepare CFC, 100 g of the milk inoculated individually and in combination was taken for estimating its antibacterial at an interval of six h. It was subjected to centrifugation in sterilized centrifuge tubes at 3000 rpm for 10 min. The sediment was discarded and supernatant was aseptically transferred to sterilized flasks and stored under refrigeration till its use.

**Preparation of inoculums:** The individual test cultures were grown in selective media broths for 24 h. Cells were harvested by centrifuging at 3000 RPM for 30 minutes at 4°C in sterile centrifuge tubes. The supernatant was discarded and the cell pellets were resuspended in sterile normal saline. This suspension was used to adjust the optical density of sterile normal saline to 0.6 at 550 nm. This will give approximately 10<sup>7</sup> cells/ml. One milliliter of readjusted culture was poured in 15 ml of selective media for the individual organisms was melted at 45±2°C.

The antimicrobial properties against test organisms were determined by cup-well assay method. The sterile plate was poured with media inoculated by test cultures was prepared. Then with the help of gel-cutter the cups were carved with diameter 7 mm. The technique was carried out under strict aseptic conditions. The cup was filled with sterile automated pipette with respective proven probiotic cell free culture filtrate along with lactic acid as a control. The plates were then incubated at 35±2°C.

## RESULTS AND DISCUSSION

Antibacterial activities of lactic acid starters: The antibacterial activity of the yoghurt culture and that of Acido-bifido-yoghurt against selected types of pathogenic

microorganisms. The test cultures of lactic acid bacteria were grown in sterilized milk. The antibacterial impact of the CFCF was evaluated against the test cultures of pathogenic organisms by employing cup-well assay. The size of well bored in the agar plates was 7 mm and control was 0.7% (LA).

### Antibacterial activity against *E. coli*

The means of data obtained from five replications on antibacterial effect of CFCF on *E. coli* is presented in Table 1. It could be seen from this data that the control well (inoculated with 0.7% Lactic Acid) shown the highest inhibitory activity. The diameter of the zone of inhibition was 13.4 indicating that a net zone antibacterial activity of 6.3 mm. Antibacterial activity observed from cell free culture filtrate (CFCF) obtained after six hour incubation of the test lactic cultures, the zone of exhibited against test pathogens *E. Coli* by *L. delbruecki* subsp. *bulgaricus* was 7.8 mm (net zone of inhibition 0.8 mm), *Str. salivarius* subsp. *thermophiles* showed 8.7 mm (net zone of inhibition 1.7 mm), probiotic culture of *L. acidophilus*-LBKV3 exhibited 7.9 mm (net zone of inhibition 0.9 mm) while *Bif. bifidum*-NCDC 255 showed the lowest impact with a zone of inhibition of 7.2 (net zone of inhibition 0.2 mm). It was observed that the CFCF obtained from the combination of all the four cultures when tested for its antibacterial activity against *E. coli* at 6-hour incubation period demonstrated the highest activity of 9.1 mm (net zone of inhibition 2.1 mm). It was further observed that the CFCF obtained from all of the test lactic cultures after incubation for 12 hour showed notable antibacterial effect against the test organism. Highest effect recorded for probiotic *L. acidophilus*-LBKV3 with a net zone of inhibition of 10.2 mm followed by Acido-bifido-yoghurt which was prepared from a combination of four culture organisms (a net zone of inhibition of 8.5 mm). The net diameter of zone of inhibition for *Bif. bifidum*-NCDC 255 was 4.2 mm and for the yoghurt cultures it was 5.2 and 3.3 mm. The control well showed 6.3 mm diameter, which was higher than the three test cultures grown individually. The CFCF obtained after

18 hour of incubation exhibit zone of inhibition was 14.7, 11.6, 23.3, 14.0, 25.8 and 13.3 mm, respectively for *L. delbrueckii* subsp. *bulgaricus*, *Str. salivarius* subsp. *thermophilus*, *L. acidophilus*-LBKV3, *Bif. bifidum*-NCDC 255, Probiotic Acido-Bifido yoghurt and the control respectively. It was seen that the CFCF obtained after 24 hrs from all lactic culture exhibit net zone of inhibition was 9.4, 5.0, 17.9, 9.4, 21.4 and 6.2 mm, respectively for *L. delbrueckii* subsp. *bulgaricus*, *Str. salivarius* subsp.

*thermophilus*, *L. acidophilus*-LBKV3, *Bif. bifidum*-NCDC 255, Probiotic Acido-Bifido yoghurt and the control respectively. It was observed that the CFCF obtained from lactic culture after 30 hour incubation showed antibacterial activity against *E. coli* with a highest net zone of inhibition of 20.3 mm in case of probiotic Acido-bifido-yoghurt, 10.4 mm in case of *Bif. bifidum*-NCDC 255, *L. acidophilus*-LBKV3 showed a net zone of inhibition of 20 mm while for yoghurt cultures the net zone of inhibition was 9 and 6.1 mm.

**Table 1: Antibacterial properties of lactic cultures against *E. coli*\***

Cultures(T)	INCUBATION PERIOD FOR PREPARATION OF CELL FREE CULTURE FILTRATES (IN HRS.)					Means (T)
	6	12	18	24	30	
	ZONE OF INHIBITION AFTER 24 Hrs, (MM)**					
<i>L. delbrueckii</i> ssp. <i>bulgaricus</i>	7.8	12.2	14.7	16.4	16.0	11.86
<i>Str. salivarius</i> ssp. <i>thermophilus</i>	8.7	10.3	11.6	12.0	13.1	11.14
<i>L. acidophilus</i>	7.9	17.2	23.3	24.9	27.0	20.06
<i>Bif. Bifidum</i>	7.2	11.2	14.0	16.4	17.4	13.24
Probiotic Acido-Bifido-Yoghurt Cultures	9.1	15.5	25.8	28.4	27.3	21.22
0.7% Lactic acid (Control)	13.4	13.3	13.3	13.2	13.2	13.28
Means(P)	9.01	13.28	17.11	18.55	19	

\*Values are the means of five replications. \*\* Including 7 mm diameter of the well

## CONCLUSION

The data of investigation on antibacterial activity of lactic cultures grown individually and in the form of Acido-bifido-yoghurt showed that Acido-bifido-yoghurt exert highest antimicrobial activity. This antimicrobial effect is probably due to synergistic effect among the lactic organism and specific metabolite produced due to metabolite. Further research is needed to elucidate the compound(s) responsible for antimicrobial activity.

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