

Original Research Article

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Antibacterial Activity of 'Dahi' Prepared from Dairy Industry, Cottage and House Hold against Food Borne Pathogens

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ABSTRACT

Dahi is highly palatable and easily digestible by human digestive system which contains macronutrients like protein, carbohydrates, fat and minerals like calcium and phosphorus. This study aims to screen the antibacterial activity of *dahi* which is good source of lactic acid bacteria (LAB). Lactic acid bacteria produce certain antimicrobial substance, which plays important role for the antibacterial activity for most of the milk products. The antagonistic properties of *dahi* against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas*, *Proteus vulgaris*, *Klebsiella* were examined using agar well diffusion method. Thirty samples of *dahi* were collected from the Amul (A1 to A10), Local market (M1 to M10) and house hold level (H1 to H10). Industrial *dahi* (collected from AMUL) and Market *dahi* were collected from local area of the Anand city. However, the spectrum of inhibition was different for the different sources of *dahi* (Dracheva *et al.*, 2007). All the samples of *dahi* exhibited the antibacterial activity against most of all the indicator pathogens. Amongst all the food borne pathogens *Escherichia coli* exhibited the highest antibacterial activity with a zone of Inhibition (ZOI) ranging from 18 to 21 mm. Industrial *dahi* showed the higher antibacterial activity against all the food borne pathogens and least antibacterial activity for *Klebsiella* (11±1 mm). *Dahi* collected from Market showed higher antimicrobial property against *Proteus vulgaris* (20 ± 2.0 mm) and least against *Staphylococcus aureus*. Whereas *dahi* collected from house showed inhibition of *Pseudomonas* (21 ± 1.5 mm) and least for *Staphylococcus aureus* like market *dahi*. All *dahi* showed the remarkable inhibitory effect against both Gram positive and Gram negative pathogenic strains.

Keywords

Dahi, Lactic acid bacteria, Food pathogen, Antibacterial activity, Zone of inhibition

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Introduction

Food is the matter of health and matter of trust also. Nowadays, food is no longer considered by consumers only in terms of taste and immediate nutritional needs, but also in terms of their ability to provide specific health benefits beyond their basic nutritional value. Currently, the largest segment of the

functional food market is dominated by healthy food products targeted towards improving the balance and activity of the intestinal micro flora. A probiotic is a "live microbial food ingredients that, when ingested in sufficient quantities, exerts health benefits on the consumer"(Doron *et al.*, 2006.). In general, it is believed that probiotics help to keep the balance between harmful and

beneficial bacteria in the gut thus maintaining a healthy digestive system. Noteworthy, there are a large number of probiotic foods which date back to ancient times which are mostly originated from fermented foods as well as cultured milk products. (Astha *et al.*, 2012)

Probiotic strains compete with other infectious bacteria for nutrients and cell-surface and helps toward them off by inhibiting their colonization. A few strains are also known to produce active enzymes which inhibit other pathogenic bacteria. The health benefits of probiotics have always been investigated with regard to their capability to sustain their availability, viability, digestibility and rendering of their health benefits to the host without altering the safety and the organoleptic properties of the food in which they have been incorporated. Today, viable probiotic strains with beneficial functional properties are available in the market as components of foods and beverages, in fermented dairy products like yogurt or as probiotic fortified foods as well as food preservatives.

As the change in taste of common people, milk is converted to many fermented products like Kefir, *Dahi*, Cheese, Butter milk, Cream. *Dahi* is highly palatable and easily digestible by human digestive system which contains macronutrients like protein, carbohydrates, fat and minerals like calcium and phosphorus (Sivakumar *et al.*, 2010). Among the various fermented milk products, international market of *dahi* has improved remarkably because of its high nutritional value and therapeutically benefits (Murugan *et al.*, 2013). Lactic acid bacteria, particularly those belonging to beneficial and non-pathogenic genera (*Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus* and *Leuconostoc*) are widely used in food industry. Among lactic acid bacteria; Lactobacilli are the most important group and are gaining increasing attention in

food fermentation industry because of their potential biotechnological interest. Lactic acid bacteria have attained significant importance in the dairy industry for their widespread use in the production of fermented foods (Farn *et al.*, 2005). This organism prevents the growth of pathogenic bacteria in different ecosystems by production of antimicrobial substance such as organic acids, hydrogen peroxide and bacteriocins. (Gopal *et al.*, 2001) The bacteriocin producing *Lactobacillus* may be used as protective culture to improve the microbial safety of foods (Olasupo *et al.*, 1997)

Materials and Methods

Collection of *Dahi* samples

Dahi samples were collected from the Amul (Amul masti *Dahi*), local market area of Anand city and from the household level.

Test organisms

The tested pathogens used were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas*, *Proteus vulgaris*, *Klebsiella*. All the cultures were maintained with regular transfer on N agar or N broth and stored in refrigerator at 4°C.

Media and reagents

The nutrient agar (NA) was used for the antimicrobial assay. The reagents, indicator, carbon substrates for biochemical tests etc. used in the study were of analytical grade and procured from Hi Media Chemicals Ltd., Mumbai, India.

Detection of antimicrobial activity by agar well diffusion method

An agar well diffusion method as described by Klaenhammer was used with some

modifications. An overnight culture of pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas*, *Proteus vulgaris* and *Klebsiella* were grown in their selective medium.

Work area was cleaned and sterilized it with 70% iso-propanol. The contents of the culture of all organisms were swirled until it was equally murky throughout. Sterile cotton swab and glass rod was used to create a lawn, or carpet, of single organism on N agar plate. A lawn of an indicator strain was made by spreading the cell suspension over the surface of N agar plates. The mouth of the test tube was flamed before and after dipping the sterile swab into it. Labelling was done at the bottom of the petri dish with name, the date, name of organism. The plates were allowed to dry and a sterile cork borer of diameter 7.0 mm was used to cut uniform wells in the agar plates. Para film was wrapped around all plates and lids were placed on it. The plates were allowed to incubate for 24 hrs in incubator at 37°C. After 24 hrs in the incubator; the presence of antibacterial activity was checked. This was done by looking for a clear area, called zone of inhibition (ZOI), surrounding a cup. To determine the affectivity of an antibiotic, diameter of the zone of inhibition around each disk was measured. Keeping the lid of the plate in place, using a ruler to measuring the diameter of the clear area in millimetres. After recording the average class data for the diameters of the zones of inhibition, The SIR (Susceptible, Intermediate and Resistant) table was used to determine whether each bacteria is susceptible (sensitive), unaffected (resistant) or somewhere in between (intermediate) for each of the antibiotics.

Results and Discussion

Comparatively, by physical and chemical observations all the three different sources of

dahi differ in their colour, smell, texture etc. resulted as per the report of Malarkannan, *et al.*, 2017. Preparation of *dahi* from the standardized or unstandardized milk leads to much variation in the total solids content of market *dahi* samples (Sarkar *et al.*, 1996).

The data consisted of zone of inhibition (in mm) for each samples of *dahi*, per bacterial species. By comparing the antibacterial activity of bacterial species, a useful measurement was found to account for this disparity in data and zone of inhibition was measured for each sample. On the basis of results obtained, as shown in Table 1.

Amul *dahi* was found to be highly effective against all pathogens except *Klebsiella*. *E.coli* was highly sensitive to any of the fermented milk product like Curd, Cheese, butter etc. (Arokiyarny and Sivakumar, 2011) However *E.Coli* and *Proteus vulgaris* exhibited the highest antibacterial activity with zone of inhibition ranging from 19 to 21 mm respectively which were similar to the result of Mahnaz Kazemipoor *et al.*, (2012) and also reported that *Lactobacillus animalis* (MF6) which exhibited the highest antibacterial activity against *E. coli* with Zone of inhibition of (20 ± 1.3) . Whereas *Staphylococcus aureus* and *Pseudomonas* were showing the susceptibility with ZOI ranging from 15 ± 1.5 mm and 17 ± 1.5 mm respectively.

As shown in Table 2, Market *dahi* exhibited highest antibacterial activity against *E.Coli* with Zone of inhibition ranging from 16.5 to 20.00 mm like Amul *dahi* but Market *dahi* had comparatively least antimicrobial activity against *Staphylococcus aureus* unlike Amul *dahi*. Mahnaz Kazemipoor *et al.*, (2012) also found that *Lactobacillus reuteri*, isolated MF15 showed greater activity against *E. coli* (12 ± 0.8 mm) and least against *Staphylococcus aureus* (8 ± 1.7 mm). It was determined that less susceptible pathogenic

bacteria to market dahi were *S. aureus* and *Klebsiella*. While similar results have been obtained from previous study of antibacterial activity of kefir on 7 bacterial species and 1 fungus was examined with disc diffusion method and it has been reported that *S. Aureus* was less sensitive pathogens and

Pseudomonas and *E. coli* were the least sensitive (Rodrigues *et al.*, 2005). Sample from (M1 to M9) also exhibited the higher antibacterial activity against *Pseudomonas* and *Proteus vulgaris* with Zone of inhibition of 18 to 20 mm and *Klebsiella* giving the intermediate result around 14 mm.

Table.1 Zone of inhibition in (mm) of samples Amul masti Dahi

Sr. No.	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas</i>	<i>Proteus vulgaris</i>	<i>Klebsiella</i>
A1	21	15	17	20	12
A2	19	15.5	18	18.5	ND
A3	20	15	18	18	ND
A4	21	16	18.5	18.5	11.5
A5	20	ND	17.5	19.5	12
A6	20	16.5	17	20	ND
A7	20.5	16	18.5	19	ND
A8	21	15.5	18	20	ND
A9	20.5	16	17.5	18.5	11
A10	20	ND	18	18	ND

Table.2 Zone of inhibition in (mm) of samples Market Dahi

Sr. No.	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomons</i>	<i>Proteus vulgaris</i>	<i>Klebsiella</i>
M1	20	19	18	18.5	14
M2	17.5	ND	19.5	18	17
M3	16.5	ND	18.5	18	14.5
M4	20	18	20	18.5	18
M5	17.5	18.5	19	20	14
M6	19	ND	19.5	19	14.5
M7	18	ND	18.5	19.5	17.5
M8	18.5	ND	20	18	14.5
M9	17	ND	19.5	19	14
M10	17.5	ND	19	18.5	17

Table.3 Zone of inhibition in (mm) of samples House Dahi

Sr. No.	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomons</i>	<i>Proteus vulgaris</i>	<i>Klebsiella</i>
H1	20	19	21	18	18
H2	21	20	20.5	19	20
H3	20.5	20.5	21	18.5	18.5
H4	20	ND	20	ND	19
H5	19.5	18	21	18.5	19.5
H6	20	ND	20.5	19	20
H7	19	ND	20	18	19
H8	19.5	18	19.5	18	18.5
H9	19.5	18.5	20	19.5	18
H10	19	19	19.5	18.5	ND

Determination of zone of inhibition, Resistant: 10 or less, Intermediate: 11-15, Susceptible: 16 or more



Figure. A



Figure. B

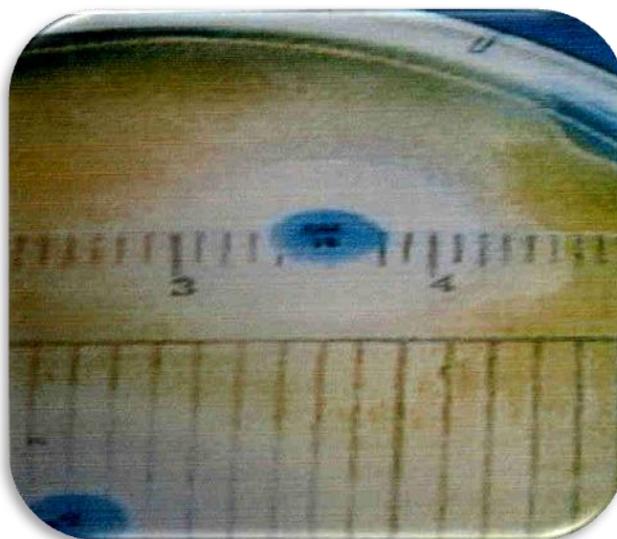


Figure. C

Figure. A: Petri Plate with a Lawn of an Indicator Pathogenic Strain of *E.Coli* and a Zone of Inhibition around the Well Containing the industrial, cottage and household *Dahi*.

Figure. B: Petri Plate with a Lawn of an Indicator Pathogenic Strain of *Pseudomonas* and a Zone of Inhibition.

Figure. C: Shows the measurement of clear area (Zone of inhibition) in mm.

As shown in Table 3, all the samples of household level (H1 to H10) exhibited antibacterial activity against all pathogens. *E. coli*, *Staphylococcus aureus* and *Pseudomonas* was more sensitive to home-made *dahi* showing Zone of inhibition of 19 to 21 mm. Whereas *Proteus vulgaris* and *Klebsiella* exhibited the antibacterial activity with ZOI ranging from 18 to 20 mm. Whatever the source of *dahi*, all the samples were more or less antagonistic against both gram positive (*Staphylococcus aureus*) as well as gram negative (*Escherichia coli*) pathogenic bacteria which is supported

by Mahnaz Kazemipoor *et al.*, (2012).

Kotz *et al.*, (1990) reported that yoghurt filtrate has in vitro antibacterial effect against three different *E. coli* strains. In this study, when inhibition zones are compared, typically, homemade yoghurt resulted in larger inhibition zones than commercial yoghurt. This may be because of making yoghurt using different strains and contamination of homemade yoghurt with different lactic acid bacteria during yoghurt making. As a result, the antibacterial effects

of *dahi* from different source fermented dairy products against common food borne pathogens showed different results according to depending on the type of fermented dairy product used, species of pathogen bacteria and time.

In case of *Staphylococcus aureus* and *Escherichia coli* there were no significant differences observed in all the three sources of the sample of *dahi*. The results of present study were in line with the earlier findings of Aslim *et al.*, (2005), that all the Samples obtained from Turkish dairy products have antimicrobials activity against *Staphylococcus aureus* and *Escherichia coli*. Amongst all, *dahi* is rich with microbial load, the results of this study were in line with the earlier findings of Riadh (2005). They reported a high incidence of microbial load in Curd (*dahi*) sample, when compared with other dairy products like Milk peda and cheese. It is however difficult to comment on the reason for this variability in the antimicrobial property amongst all the different sources of *dahi* since each one has different procedure of preparation.

In conclusion, the results obtained from study demonstrated the remarkable antimicrobial attributes of the *dahi* prepared from different fermented product of milk. The highest antibacterial effect against food borne pathogens used in this study was seen in different sources of *dahi*. A large number of lactic acid bacteria present in food products with different bioactive potentials especially in the form of antimicrobial properties have been identified from a variety of dairy products and also from a variety of plant sources mostly in the form of fermented and pickled vegetables. These scientific evidences have been a motivating factor to choose a dairy based fermented product, which could further confirm the results of this study, On the other hand, such positive outcomes would

be a leading point towards application of simple worthy traditional methods such as fermentation.

For good health of all consumers, strict and preventive measure should be adopted to ensure contamination free milk products. Good hygienic, sanitary condition, good manufacturing practise and good storage condition will reduce the risk of poor microbiological quality and increase the safety of the product. It is suggested that the probiotic bacteria or/and metabolites in food can be used as an antibacterial agent against to food borne pathogens alone or in various combinations.

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