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ESTIMATION OF MOST EFFICIENT PROBIOTIC CONTAINING MILK FROM SELECTED MILKING BREED.

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Abstract: Milk has many probiotic properties which are beneficial as for human or animal health is concerned. Some of the organisms that are having probiotic properties present in milk such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, etc. *Lactobacillus* which has many probiotic properties is most commonly found in milk samples of any milking breed. A probiotic strain of *lactobacilli* can prevent the development of a variety of range of animal and human pathogens. The motive of this study is to observe the most efficient probiotic properties of probiotic bacteria from different variety of selected milk breeds. Where the isolation of pure culture was obtained by continuous sub-culturing. Identification of LAB by observing the morphological characteristics, biochemical test, etc. This isolated pure culture was examined for further studies including the activity of that LAB against the enteric pathogens, tolerating capacity of bile salt, sensitivity to the different antibiotics, tolerance to the acidic pH, etc. In this series of tests, the desired colonies were isolated after pure culturing from a variety of selective breed milk like buffalo, goat, and cow milk. From this study, we find out the potential and nutritional properties of milk for future prospectsct.

IndexTerms **- Isolation, Probiotic, Lactic Acid Bacteria, Efficient**

I. INTRODUCTION

The term Probiotic is first coined by the Russian zoologist and Nobel Prize holder Elie Metchnikoff [1]. The definition of probiotics is the live microbial culture that can beneficially affect the host and easily survive in the human or animal body like a normal microbiota. [2] Probiotics are generally nondigestible food that is beneficial for the host by stimulating the growth of microflora which is good for human health exist in the gut. [3] The probiotics are commonly found in curd, buttermilk, cheese, etc. [4] Probiotics also found in some kinds of food that is manufactured by bacterial fermentation are sauerkraut, tempeh, kimchi, chocolate, Japanese miso, bread, beer, olives, sourdough, and pickles, etc. [5] Among that the consumption of milk including these probiotic-rich products is the daily diet helps to keep the digestive system work properly and smoothly as well as it improves natural defense of the body [6]. Important characteristics of ideal probiotics are the organism should be non-toxic and non-pathogenic in nature [7]. They should be able to survive in normal gut conditions such as high concentration of bile salt and high acidic condition [8]. They should show antimicrobial activity against enteric pathogens, and also have to show resistance against different antibiotics [9]. This quality shows the potency of the probiotics [10]. The milk sample from different milking breed water, fat, minerals, carbohydrates, proteins contents which are in the different concentration and has the different probiotic efficiency [11]. Applications of probiotics includes enhancement of epithelial barrier function, as a preventive measure for allergic reactions, it helps to reduce serum cholesterol, it is used for prevention of dental caries formation, it helps to prevent from respiratory infections and many more applications are there [12].

II. MATERIALS AND METHODS:

Sampling

The freshly collected raw milk sample of three different milking breeds such as goat, Gir cow, and buffalo was taken from the local farm of the vicinity of Kopargaon, Maharashtra.

Screening and Isolation of bacteria from milk samples

After sampling milk from the different animals, the milk sample was subjected to serial dilution in sterile saline solution from 10^3 to 10^5 [13]. Then 100 ul of the sample was taken from the last dilution and spread on the surface of MRS agar medium as MRS is the selective medium for the isolation of lactobacillus spp. Then plates were kept for overnight incubation for 24 hrs. the same procedure was repeated all different milk samples. After overnight incubation colonies on the plates were observed CFU count was estimated [14][15].

Morphological and biochemical characterization of organisms

Colony morphology was studied by observing selected colonies. The biochemical characterization was done by following the Starch hydrolysis test, Citrate hydrolysis test, Catalase test, Methyl red test, Casein hydrolysis test, Urease hydrolysis test, Vogous proskar test, Indole test, Gelatin hydrolysis test, Carbohydrate fermentation test, Mannitol fermentation test, and Salinity test, etc. [16][17].

Analysis of probiotic properties

Detection of antimicrobial activities against selected pathogens

The antimicrobial properties of isolated Lactic Acid Bacteria species were determined by performing the disc diffusion method. Sterile Whatman No.1 filter paper discs, (10mm) were immersed into the broth of isolated bacterial culture of Lactobacillus spp. inoculated for 48 hours and then sited on solidified Nutrient Agar spread with 3h old culture of test pathogens, which is *Escherichia coli*. The plates were stored at 4°C for 1h diffusion and then incubated at 37°C for 24 hours. After the incubation, the zone of inhibition will measure [18].

Acid tolerance and bile salt tolerance

The isolated probiotic bacterial spp. was inoculated into MRS broth for verification of pH, i.e., pH 2, 3, 4 and 5; and the broth as well with different concentrations of bile salt i.e., 0.5, 1.0, 1.5 and 2.0% and incubated at 37°C for 48 hours. 0.1ml inoculum was then transferred to the MRS agar plate by using the pour plate method and incubated at 37°C for 48 hours. The growth of Lactic Acid Bacteria on the MRS agar plate was used to find the isolate as Acid tolerating and Bile salt tolerating bacteria [19] [20].

Antibiotic susceptibility test against selected antibiotics

A suspension of freshly grown test organism i.e., *E. coli* was mixed with 5ml of MRS soft agar (0.5% agar) and overlayed on bottom layers of MRS agar. The antibiotic resistance of isolated LABs was apprised using antibiotic discs of various antibiotics such as Streptomycin, Penicillin, Ampicillin, and tetracycline were aseptically placed on MRS agar plates [21].

III. RESULT AND DISCUSSION:

Table :1 CFU/ ml for the different milk samples

Sample	Gir cow milk	Goat milk	Buffalo milk
Sample1	37×10^{-5}	40×10^{-5}	35×10^{-5}
Sample2	41×10^{-5}	36×10^{-5}	37×10^{-5}
Sample3	39×10^{-5}	38×10^{-5}	38×10^{-5}
Average CFU/ml	38×10^{-5}	39×10^{-5}	36×10^{-5}

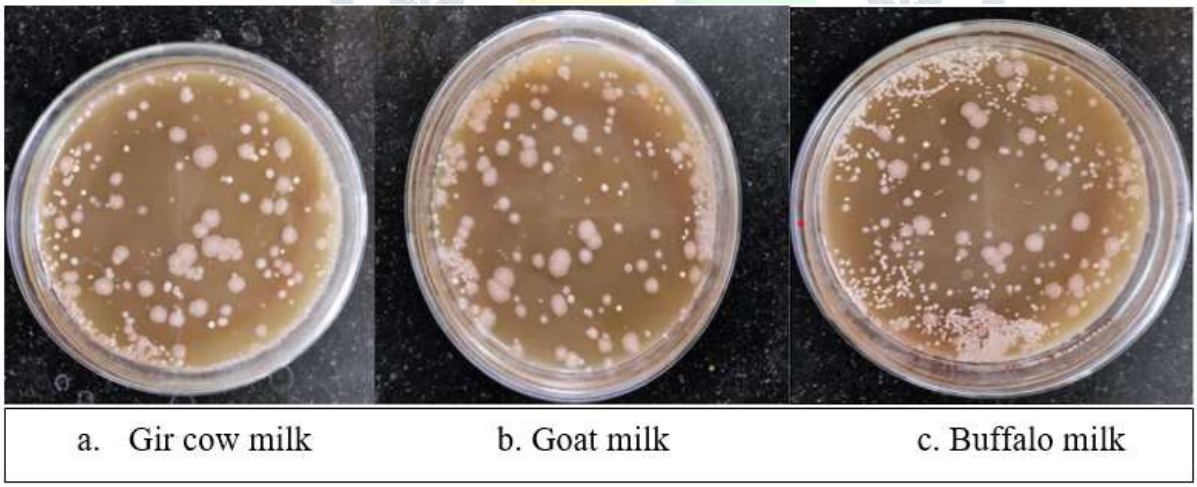

Figure 1. Screening of Bacterial Present in Gir Cow Milk, Goat Milk, and Buffalo Milk, Showing Growth on MRS Media Plates

After overnight incubation plates with respective milk, samples were observed and the average CFU/ ml count was calculated. It was found that the average CFU count for milk sample from Gir cow was 38 ×10-5, then for goat milk and buffalo milk average CFU count was 39 ×10-5 and 36 ×10-5 respectively.

Table 2. Morphological and Biochemical Characterization of screened Probiotic Bacteria from different milk samples.

Isolated colonies / Characters	(Isolate I) Gir Cow	(Isolate II) Goat	(Isolate III) Buffalo
Size	1mm	1mm	1mm
Shape	Circular	Circular	Circular
Colour	Yellow	Off white	Orange

Margin	Entire	Entire	Entire
Elevation	Convex	Convex	Convex
Opacity	Opaque	Opaque	Opaque
Consistency	Smooth	Smooth	Smooth

Biochemical test for selected isolated colonies

Indole test	+	+	+
Methyl red test	+	+	+
Vogous proskers test	-	-	-
Citrate utilization	-	-	-
Sugar utilization	-	-	-
Sugar fermentation	-	-	-

Carbohydrate test:

Lactose	+	+	+
Dextrose	-	+	-
Sucrose	+	+	+
Mannitol	-	-	-

Enzyme test:

Catalase	+	+	+
Oxidase	+	+	+

Analysis of probiotic properties

Detection of antagonistic (antimicrobial) activities against selected pathogens

The antimicrobial activity of lactobacillus was determined by the agar well diffusion method. The antimicrobial activity is determined against some pathogenic strains such as *E. coli, S. aureus* which is basically part of the normal gut flora and these organisms are also known as enteric pathogens.

Figure 2. Zone of inhibition observed around the wells, well no 1 & 2 contains Goat milk sample, well no. 3 Contains Buffalo milk sample and well no. 4. Contains Cow milk sample on MRS media containing plate pre-inoculated with *E. coli* suspension.

In the diagram given above, the MRS agar-containing plate is spread with the suspension of *E. coli* which is an enteric pathogen, and the wells on the agar plate are filled with a suspension of different isolates from milking breeds. The numbering given to wells for the differentiation of isolates as well no. 1 & 2 is inoculated with samples from Goat milk, well no. 3 is inoculated with Buffalo milk and well no. 4. Is inoculated with Cow milk.
After 24 hours of incubation, a zone of inhibition is observed around the wells against the enteric pathogen *E. coli.*

Antibiotic resistance test against selected antibiotics

The resistance to the antibiotics by the lactobacillus was determined by the disc diffusion method.

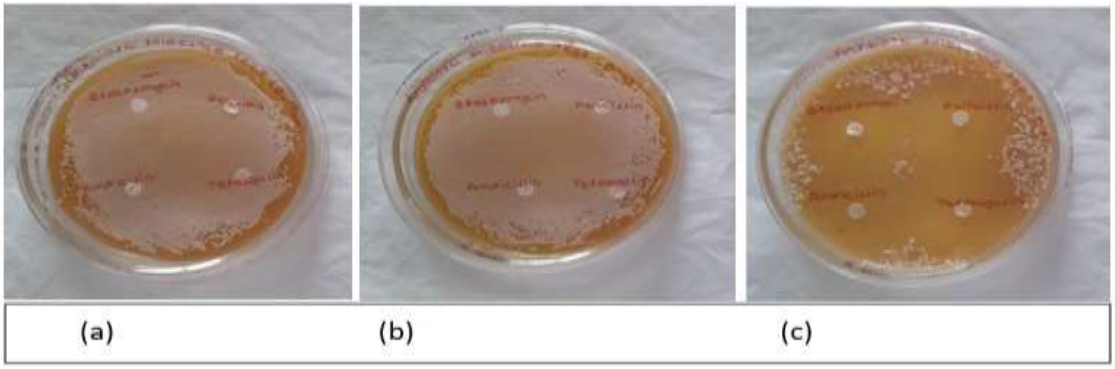

Figure 3. MRS media plates inoculated with a) Buffalo milk sample b) Goat milk sample c) Gir Cow milk sample. The bacteria present in Goat milk show more resistance to the antibiotics as compared to Buffalo milk and Gir Cow milk.

MRS agar plate spread with the organism isolated from milk sample of Buffalo which is then introduced to 4 different antibiotics such as Streptomycin, Penicillin, Ampicillin, and Tetracycline by disc diffusion method. No zone of inhibition is observed hence the isolated organism is able to survive in the presence of antibiotics.

MRS agar plate spread with the organism isolated from milk sample of Goat which is then introduced to 4 different antibiotics such as Streptomycin, Penicillin, Ampicillin, and Tetracycline by disc diffusion method. No zone of inhibition is observed hence the isolated organism is able to survive in the presence of antibiotics.

MRS agar plate spread with the organism isolated from milk sample of Gir Cow which is then introduced to 4 different antibiotics such as Streptomycin, Penicillin, Ampicillin, and Tetracycline by disc diffusion method. A zone of inhibition is observed hence the isolated organism is unable to survive in the presence of antibiotics.

Acid and bile salt tolerance:

The acid tolerance capacity of the probiotic bacteria is observed by inoculating it into the MRS broth with different acidic conditions such as pH 2, 3, and 4 after 24 hours of incubation at 37°C. After incubating for 24 hours, the growth of probiotic organism is observed by checking the absorbance at 560 nm by using a UV spectrophotometer. More turbid broth shows more absorbance, hence more capacity to tolerate acidic conditions.

Table 3. The growth of probiotic organisms is observed by checking the absorbance at 560 nm by using UV spectrophotometer

Isolates	Control	(Isolate I) Gir Cow	(Isolate II) Goat	(Isolate III) Buffalo
Absorbance at 560 nm	00	0.24	0.30	0.26

Bile salt tolerance capacity of the probiotic bacteria by observing the bacterial growth on MRS agar plates with bacterial suspension of different bile salt concentrations for 24 hours at 37°C. The plate of probiotic bacteria from the goat milk sample shows comparatively more growth of organisms on bile salt containing MRS agar plate.

IV. CONCLUSION:

Isolated Lactic Acid Bacteria species give the exposition to the good and essential probiotic characteristics and can therefore be used for dairy or food fermentations and give the vital contribution in benefits to consumers as per as the health is concern. These lactobacilli can take part in stabilizing the gut microbiota and plays an important role as a permeability barrier in the intestine, thereby encouraging the immunological barrier to the gut mucosa. They also show the major significance in the prevention and treatment of medical conditions related to the lesser functions of the gut mucosal barrier and assisted inflammatory responses. The probiotics perspective is acceptable for redesigning the natural condition by restoring an insufficiency caused by the inclusion of foreign chemicals into the body, e.g., compromising subsequent therapy and antibiotics. The preventive and therapeutic purpose of controlling intestinal infections could be fulfilled by antibiotic-resistant strains of probiotics.

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