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Antibiotic Susceptibility of pro-biotic lactic acid bacteria (LAB) strains (bifidobacterium and lactobacillus casei) isolated from raw and fermented camel milk

JV'n N.s Malini1, JV'n Prof. (Dr) Pramod K. Raghav, JV'n Dr. Khushbu Verma2*
Faculty of Education and Methodology,
Jayoti Vidyapeeth Women's University, Jaipur Rajasthan
Corresponding Author Email id- propresident@jvwu.ac.in; pramodraghav31@gmail.com

Abstract :

In the aim of thestudy, the potential probiotic properties of Lactobacillus casie CM1 and bifidobacterium CM2isolated from raw camel milk and traditional Raw and fermented camel milk, respectively, were studied. The probiotic properties of isolates that were investigated included the hemolysis, antibacterial activity, antibiotic resistance, low pH and bile salts activity, survival under simulated Gastrointestinal Tract conditions. None of the isolates exhibited hemolytic activity. They were susceptible against (amoxicillin/25 ?g, penicillin /30 ?g, cefoxitin/30 ?g, bacitracin/30 ?g, chloramphenicol/30 ?g, polymyxin /30 ?g, gentamycin/30 ?g, neomycin/30 ?g, vancomycin/30 ?g, polymyxin B/30 ?g. Lactobacillus casie CM1 and bifidobacterium CM2 (P<zero.05) retained their viability at pH 3.0more than 7.0 log CFU mL-1and under simulated GIT conditions. Both isolated LAB have MICs of 6.26 to 25 mg mL-1 against pathogenic bacteria. They exhibited capacity to adhere to hydrocarbon (xylene), and possessed a high auto-aggregation and co-aggregation rate (more than 40%).

Keywords : Lactobacillus casie CM1, bifidobacterium, LAB, auto-aggregation coaggregation rate

1. INTRODUCTION :

Camel milk has been consumed in numerous parts of the world, either fresh or fermented. Camel milk is regarded as one of the primary sources of dairy products for human consumption in dry climates, with possible medicinal benefits. Recent studies have shown that camel milk is a natural source of probiotics. LAB is a potential source of biological resources for dairy technology. Due to its crucial function in the majority of fermented foods and its capacity to produce different antimicrobial compounds that support probiotic properties, such as antitumor activity, lowering serum cholesterol, relief of lactose intolerance, immune system stimulation, and stabilisation of the intestinal microflora, LAB is currently the subject of intense international research. Lactic acid bacteria strains that generate exopolysaccharide are utilized to improve the texture and viscosity of fermented milk. Traditional dairy products are typically maintained through spontaneous fermentation. Modern large-scale manufacturing procedures, on the other hand, often include established strain starting systems to ensure consistency, safety, and quality in the end result. Camel milk's beneficial microbiota, particularly lactic acid bacteria (LAB), is a probiotic potential source of biological materials for use in dairy technology (Jans et al., 2012; Khedid et al., 2009). However, camel milk must be transformed by fermentation, and further study is required to understand the method (Fguiri et al., 2015). This research would benefit from the full description of the normal LAB community given in this paper.

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Among the different milk preservation processes, fermentation is frequently used by the dairy industry to produce infection-free products. Camel milk, both raw and fermented, is linked by culture and custom. Lactic acid bacteria, one of the most extensively employed microorganisms, serve a significant role in the preservation of milk and dairy products. Their common presence in food, combined with their historical use, leads to their acceptability as generally Recognised as Safe (GRAS) for human consumption, and they have been accepted for Qualified Presumption of Safety (QPS) (Liu et al., 2011, Jans et al., 2012). The primary genera of BAL are Lactococcus, Lactobacillus, Pediococcus, Leuconostoc, Streptococcus, Enterococcus, and Bifidobacterium (Liu et al., 2011). Before bifidobacteria and lactobacillus casei strains may exercise their stated benefits in the intestines, they must first meet numerous criteria and survive the harsh circumstances of the gastrointestinal tract, which include acidic stomach conditions and bile salts. Probiotics must survive in the intestines in order to provide health advantages to the host. Gastric fluids, digestive enzymes, and bile salts found in the stomach and intestines offer a hazard to these bacteria. Lactobacilli and bifidobacteria survive the stomach and small intestine and reach the large intestine in high numbers, where they thrive and cling to the mucosa of the intestinal tissues.

The raw camel milk microbiome is a key component of many traditional fermented dairy products, including cheese. Similar tendencies were seen in the quantitative progression of lactococci, lactobacilli, leuconostoc, and enterococci from camel milk to finished products such as cheese. Traditional fermentation employs LAB-dominated microflora, most likely originating from the container's surface (Jan's et al., 2012). Traditional foods such as camel milk have been investigated as natural sources in Europe, Africa, and other parts of the world. However, research into the isolation of active LABs from camel milk is limited or non-existent. As a result, the current study was designed to identify and characterize active LAB from raw camel milk.

2. MATERIAL AND METHODS :

2.1 Maintenance of samples : The culture was grown in MRS broth medium at 37° C for 48 hours, culture has inoculated at a concentration of 1% and 2% in skim milk and incubated at 37 ° C for 48 hours, the incubation maintains the optimal temperature for growth and results in curdling.

2.2 Medium compositions : All medium components, The analytical grade medium components and prepared media used in the study were purchased from Hi-media (India), Merck (India), and Sigma Chemical Company (USA). The media were prepared in double distilled water and sterilized by autoclaving at 15psi 15 minutes except skim milk, which was sterilized at 12psi for 15 minutes.

2.3 Isolation and identification of LAB : Six Camel milk samples have been collected in sterilized bottles from Jaipur, Rajasthan. To 90 mL of sterile NaCl solution (0.85% w/v), 10 mL of raw milk and fermented milk samples were added. After homogenising the suspensions with a vortex, 100 L of a suitable dilution (10-1 to 10-3) was spread on MRS agar. Anaerobically, the plates were incubated at 37° C for 48 hours. after the incubation period, the isolated bacteria have been selected for molecular identification based on Gramme staining, catalase activity, and oxidase activity, acidifying activity, lipolytic action, antibacterial effect, and tolerance of the isolated bacteria to acidic pH as described by (Gotcheva et al. 2002).

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2.4 Amplification and Sequencing of PCR bands : Agarose gel electrophoresis was performed by DNA fragments and to excise corresponded bands with a sterile scalpel by using UV light after ethidium bromide staining and the amplicons of PCR were purified and stored at -20°C. The National Centre for Biotechnology Information carried conducted database searches for similar sequences using BLAST to determine the closest recognized relative species after sequencing was completed.

2.5 Antibiotic Resistance : The diffusion method was used to determine the antibiotic susceptibility of the intestine and the viable cell counts were done on MRS agar (DeMan, Rogosa and Sharpe) to observe the survival rate.10 antibiotics were selected as samples of the different types of important antibiotics amoxicillin, penicillin, cefoxitin, bacitracin, chloramphenicol, polymyxin, gentamycin, neomycin, vancomycin.

Figure No: (1) Antibiotic susceptibility of the identified LAB was determined by using the agar well diffusion method

3. RESULTS AND DISCUSSION :

3.1 Molecular Identification of LAB Isolated From Camel Milk :

Lactobacillus casie CM1 and bifidobacterium CM2 isolated from raw and fermented camel milk with the highest percentage of identity (97%) were chosen for further study based on BLAST results of the 2 isolates. Lactobacillus casei, L. acidophilus, and L. plantarum were identified from raw camel milk, according to the study. The genera Enterococcus, Lactobacillus, and Lactococcus were prominent in raw and fermented camel milk, and 30 isolates were identified using 16S rRNA gene sequences. According to the findings, non-hemolytic activity is the first attribute of a selective probiotic strain, indicating that it does not contain harmful bacteria. When red blood cells were cultivated in blood agar, none of the selected isolates were capable of hydrolyzing.

3.2 Antibiotic Susceptibility of LAB : Based on the results Lactobacillus casei CM1 is resistant to decrease ($P< 0.05$)in bile salts in comparison to bifidobacterium CM2, however, both of them retained their viability more than 7.0 log CFU mL-1 and the results are consistent with the results of Nami et al. (2014), (Lee et al., 2015), and Bian et al. (2016).The isolated LAB had MICs ranging from 6.26 to 25 mg mL-1 against Gram-positive and Gram-negative pathogenic bacteria. The isolated LAB had MICs ranging from 6.25 to 25 mg mL-1 against Gram-positive and Gram-negative pathogenic bacteria. CFS MIC values evaluated by LAB exhibit a broad spectrum of anti-pathogenic activity. When the (CFSs)

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were neutralised, they had no bactericidal effect. It is possible to deduce that the antibacterial action of the (CFSs) is pH dependent. LAB strains' antimicrobial activity was attributed to the formation of organic acids, bacteriocins, and other metabolites (Abushelaibi et al., 2017).

Antibiotic susceptibility testing was performed on the two LAB, and the majority of the 10 antibiotics tested reduced their growth to some extent (Table No 1-2). Interestingly, three antibiotics (bacitracin, vancomycin, neomycin, gentamycin, and penicillin) inhibited all of the tested strains with inhibition zones of neomycin-41mm, bacitracin-39mm, gentamycin-38mm, vancomycin-38mm, and penicillin-37mm (14.0 0.0,- 28.5 0.71, 9.5 0.71 - 40.0 0.0, and 9.5 0.71). on the other hand Cefoxitin, amoxicillin, chloramphenicol, ciprofloxacin, and polymyxin, had a inhibition zones of cefoxitin-34mm, amoxicillin-36mm, chloramphenicol-33mm, ciprofloxacin-30mm, and polymyxin-36mm diameter, respectively, at lower levels of 89%, 73.9, and 67.4% inhibition effect on the tested strains. Only four antibiotics-amoxicillin, cefoxitin, polymyxin, and chloramphenicol-had modest inhibitory action at levels of 17.4%, 30.4%, 34.8%, and 45.7%, respectively.

Table No: (1) : Inhibition zone of Antibiotics with Lactobacillus strains

S.No.	Antibiotics	Concentration	Inhibition zone Diameter	L.casei	BN11
1	Bacitracin	/30 ?g	39mm (14.0±0.0)	++++	++++
2	Vancomycin	/30 ?g	38mm (28.5±0.71	++++	++++
3	Neomycin	/30 ?g	41mm (9.5±0.71)	+++++	++++
4	Gentamycin	/30 ?g	38mm (40.0±0.0)	++++	++++
5	Penicillin	/30 ?g	37mm (9.5±0.71)	++++	++++

The profile of the selected LAB (n = 2) for main probiotic characteristics and the antibacterial activity No inhibition, +: inhibition zone 8.0-10.0mm, ++: inhibition zone 10.1-15.0mm, +++: inhibition zone 20.0-30mm, ++++: inhibition zone 30-40mm.

Table No: (2) Inhibition zone of Antibiotics with Lactobacillus strains :

S.No	Antibiotics	Concentration	Inhibition zone Diameter	L.casei	BN11
1	Cefoxitin,	/30 ?g	34mm (15.0±1.41)	++++	++++
2	Amoxicillin,	/25 ?g	36mm (40.0 ± 1.41)	++++	++++
3	Chloramphenicol	/30 ?g	33mm (11.0 ± 1.41)	++++	++++
4	Ciprofloxacin	/30 ?g	30mm (23.5 ± 0.71)	++++	++++
5	Polymyxin	/30 ?g	36mm (9.5 ± 0.71)	++++	++++

The antimicrobial activity of Lactobacillus casei, isolated from poultry waste and bifidobacterium, isolated from camel milk were attributed to the production of organic acids such as lactic and acetic acid. There is a hypothesis that organic acids, by neutralizing the cytoplasmic membrane's electrochemical potential, increase the membrane strains. The ability of a strain to adhere to hydrocarbons is

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characterised as cell surface hydrophobicity (Collado et al., 2008). In L. acidophilus CM6, there was a correlation between auto aggregation and adhesion capacity, as well as a relationship between adhesion and hydrophobicity (Del Re et al., 2000) parameters in selected Bifidobacterium strains.

CONCLUSIONS :

In the prevailing study, Bifidobacterium CM2 and Lactobacillus casei CM1 that were isolated from raw and fermented camel milk, respectively, displayed antibiotic susceptibility and no hemolytic activity. Isolates are therefore regarded as safe potential probiotics. Both LAB isolates shown a broad spectrum of antibacterial activity. The tested LAB showed a lot of potential for adhesion as well as co- and auto-aggregation. These traits are connected to an isolate's capacity to attach to intestinal epithelial cells and compete with pathogens. Therefore, we draw the conclusion that the probiotic cultures Lactobacillus casei CM1 and bifidobacterium CM2 possess all the essential probiotic qualities needed to be used in the creation of functional dairy.

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