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ORIGINAL ARTICLE

Antagonistic activity of Lactic Acid bacteria isolated from Human colostrum against common human pathogens

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ABSTRACT
The present study was carried out to isolate those probiotic strains of lactic acid bacteria (LAB) from human colostrum that possess antibacterial activity against wide range of human pathogens. All the isolates were biochemically characterized and studied for their probiotic properties including acid tolerance, production of bacteriocin and hydrogen peroxide, tolerance to different concentrations of bile salts and resistance against common antibiotics. Antibacterial activity of the isolated species was tested against different Gram's negative as well as positive pathogens including Klebsiella pneumoniae, Proteus vulgaris, Staphylococcus aureus, Salmonella typhi, Escherichia coli and Pseudomonas aeruginosa. All isolates showed inhibitory effects against above mentioned bacteria. L15002 showed high antibacterial activity against all test pathogens. It was genotypically confirmed as Lactococcus lactis. The growth of bacteria was assessed in vitro using viable bacterial counting. Antibacterial response of Lactococcus lactis was attributed to the production of bacteriocins which were responsible for cell death as well as cell inhibition. The present study is focused on antagonistic activity of different species of LAB isolated from human colostrum.
Keywords: *Lactic acid bacteria, Probiotics, Bacteriocins, Antagonistic activity.*

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INTRODUCTION

Human milk is the gold standard for infant nutrition as it contains multiple biologically active components, including immune factors, lipids, oligosaccharides, microRNAs hormones and various other nutrients in different quantities [29, 17]. It promotes the infant growth and development, and WHO has recommend exclusive breastfeeding during the first 6 months of life [35]. Microorganisms have emerged as important bioactive components of human milk that play an important role as commensal, mutualistic or potentially probiotic bacteria in healthy human milk [12], which has created increasing interest in human milk microbiology and their effects in maternal-infant health. Species of *Lactobacillus salivarius, Lactobacillus fermentum, Lactobacillus gasseri, Bifidobacterium breve, Bifidobacterium adolescentis*, and *Bifidobacterium longum* subsp. *infantis* have demonstrated potential to promote mother and infant health, including the prevention or treatment of lactational mastitis, [13, 14, 32].

Antibacterial effects of lactic acid bacteria (LAB) against human pathogens are well documented in scientific literature where these have been isolated from different milk laying animals such as buffalo, cow, goat and camel milk [8]. Previous studies have reported the antagonistic activity of *Enterococcus* spp., *Pediococcus* spp., *Lactobacillus* spp and species of *Lactococcus* [20]. LAB have high potential of probiotic properties that are generally acceptable for human consumption [10]. LAB have high industrial importance in fermentation and pharmaceutical industries due to their wide range of applications [24]. They play a significant role in developing immune system of infants [11]. Large number of bacteria with high probiotic potentials enter infant's gut through breast feeding in initial days of life [30] that protects the infant immune system in fighting against wide variety of pathogens [34]. They are very commonly reported in the gut of adults [22]. Problems related indigestion are attributed to the imbalance of gut

microbiota [19]. LAB produce large amounts of other inhibitory substances like different bacteriocins, lactocins and some other organic acids [17, 18, 25, 15] inhibit the growth of other microorganisms. Mechanism of microbial antagonism is attributed to the competition for nutrients, space, secretion of inhibitory substances, stimulation of immune system and adhesion inhibition of pathogens to mucosal surface and various other factors [34, 21]. The rapid increase in emergence of chronic toxicity caused through drug-resistant strains has encouraged the researchers for developing an alternate treatment for controlling and treating bacterial infections [36, 37]. This papers deals with the antagonistic activity of LAB against common human pathogens with the objective to find out their probiotic potential.

MATERIAL AND METHODS

Colostrum samples were collected from the maternity ward of JNU hospital, Jaipur after approval from institutional ethics committee. Samples were serially diluted in sterile peptone water and 0.1mL of inoculum was aseptically inoculated on MRS (deMan, Rogosa & Sharpe) agar plates. The plates were incubated anaerobically at 37°C for 24-48h. Isolates were sub-cultured to get pure colonies and were identified phenotypically using different biochemical tests. Identification and biochemical tests were carried out at Shobhit Institute of Engineering & Technology, Meerut. Probiotic properties of isolates were checked using different tests as previously mentioned in [26-28].

Test organisms

All six strains of pathogens used in the present study are already available in our laboratories. The indicator pathogens used in the study were *Staphylococcus aureus* ATCC25923, *Proteus vulgaris* ATCC33420, *Escherichia coli* ATCC25922, *Klebsiella pneumonia* MTCC3384, *Salmonella typhi* MTCC733 and *Pseudomonas aeruginosa* ATCC27853. All six pathogens were overnight grown in nutrient broth at 37°C.

Anti-bacterial activity of LAB

The antagonistic activity of isolates against test pathogens was measured using agar well diffusion method. The pathogens were inoculated on Mueller Hilton agar plates and with the help of sterile cork borer (6 mm in diameter), agar surface was punctured to create wells. Liquid cultures of isolates grown overnight in MRS broth were poured in the wells with the help of micropipette. The plates were allowed to incubate at 37°C for 24-48h in upright position. Zones of inhibition were observed and measured using zone measuring disc after completion of incubation [5, 7].

RESULTS AND DISCUSSION

Eight isolates which were showing best probiotic properties were screened for testing the antagonistic activity against common human pathogens. These isolates were morphologically and biochemically identified using Gram's staining, catalase test, oxidase and sugar fermentation tests as recommended in Bergey's Manual of Systematic Bacteriology and earlier described by Bisht and Garg [7]. All the 8 isolates were Gram's positive, catalase and oxidase negative in nature. On the basis of their ability to ferment different sugars, genus of the isolates were identified that included 3 isolates of *Lactobacillus*, 3 of *Lactococcus* and 2 were *Pediococcus*. All the eight isolates were tolerant to acidic pH, and bile salts and were also resistant to different antibiotics. Antagonistic test revealed that isolate (I4601) was showed highest antibacterial activity against *Staphlococcus aureus* ATCC25923 (Fig 1) , *Proteus vulgaris* ATCC33420 *Escherichia coli* ATCC25922 (Fig 1) , *Klebsiella pneumonia* MTCC3384 while *Salmonella typhi* MTCC733 *Pseudomonas aeruginosa* ATCC27853 were inhibited by isolate no I0502. The isolate (I4601) isolate was identified as *Lactococcus lactis* (Table 1).

Table 1: Zone of inhibition of selected lactic acid bacteria

Isolate Name	*Staphlococcus aureus ATCC25923*	*Proteus vulgaris ATCC33420*	*Escherichia coli ATCC25922*	*Klebsiella pneumonia MTCC3384*	*Salmonella typhi MTCC733*	*Pseudomonas aeruginosa ATCC27853*
Zone of Inhibition (mm)						
I2501	16mm	5mm	8mm	5mm	8mm	10mm
I3302	19mm	6mm	10mm	04mm	09mm	10mm
I4601	24mm	8mm	12mm	09mm	16mm	13mm
I0502	21mm	7mm	14mm	06mm	18mm	18mm
I5201	21mm	8mm	10mm	04mm	14mm	16mm
I5601	23mm	6mm	06mm	07mm	10mm	10mm
I0601	20mm	7mm	09mm	06mm	06mm	09mm
I5502	18mm	5mm	07mm	05mm	06mm	08mm

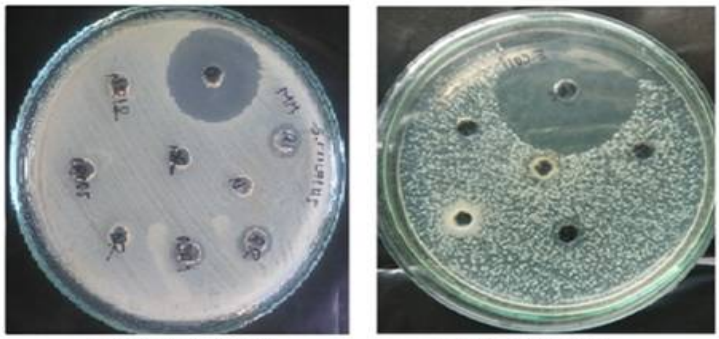

Fig 1: Antagonistic activity of *Lactococcus lactis* against *Staphylococcus aureus* and *Escherichia coli*

The scientific interest in studies on human milk bacteria is mainly focused on (a) the roles that milk bacterial communities play on infant health and development, (b) their influence on the colonization of the gut microbiota in early life; (c) the roles that they may play on maternal health, including breast health; (d) the maternal, infant, environmental and medical factors that shape and modulate their compositions, (e) their antagonistic activity with different enteric pathogens (f) origin, in order to confirm or refuse the existence of an endogenous oral-entero-mammary route allowing the selective translocation of some bacteria from the maternal digestive tract to the mammary gland, (h) differential microflora of normal or scissor deliveries and (i) impact of diet on composition of microflora of human colostrum.

The present investigation aimed to isolate and characterize human colostrum bacteria and to find out their antagonistic activity against common human pathogens. We identified 3 isolates of *Lactobacillus*, 3 of *Lactococcus* and 2 of *Pediococcus* on MRS agar medium under anaerobic/partially aerobic conditions. In view of the **a**ntagonistic activity of LAB and its commercial applications in food industry [3, 4, 6, 12] the microbiologists are isolating potentially antagonistic LABs from different sources and evaluating their antimicrobial activities against common food borne human pathogens. Almedia *et al.,* [1] have recently characterized a large number of functional and taxonomic groups of human gut microbiota and have presented a Unified Human Gastrointestinal Genome (UHGG) collection comprising 204,938 non-redundant genomes from 4,644 prokaryotes which encode >170 million proteins and have highlighted the importance of studies on gut microbiome. Roughly 40 trillion microbes, consisting of more than 3000 species of several viruses, bacteria, archaea, fungi and other eukaryotes live in and on our body and are extremely important for our health, however, few cause diseases and disorders [33]. Microbial biomass in/on our body consists of 1 kg (approximately) representing about 1000,000 genes while entire human genome consists of approximate 30,000 genes and the human microbial genome greatly influence the physiology and behaviour of human. Gut microbiome is the principal ecological niche and secretes various enzymes that are mainly responsible for the digestion of food and also accounts for 70% of the total immunity of the body. Lactic acid bacteria (LAB) constitute the major part of gut microbiome but these are difficult to culture because of the requirement of anaerobic / partially aerobic conditions for their culture. Most scientists have focused on genomic characterization of gut microbiota and a large number of microbial species have been identified [31]**,** however, very few species have been cultured so far, mainly because of the problems of quick death of gut microbes in between the sampling and processing.. The isolation, characterization and culture of 8 isolates of LAB from human colostrum as representative of gut microbiota of human in this paper suggest that gut microbes if cultured under anaerobic / partially aerobic conditions on MRS agar media, can help in isolation of more and more species which can then be evaluated for their commercial exploitation. Diet influence the gut microbiome [2] and the importance of plant based diet in regulation of gut microbiome and its impact on human brain functions has been beautifully reviewed by Medawar et al. [23]. Good bacteria can also help to manage depression [9]. It is suggested that human gut microbiome need to be cultured so that their probiotic value may be evaluated and used for commercial purposes. The presence of good bacteria in human colostrum having antagonistic activity suggest that breast feeding to infants provide protection to infants from enteric pathogens and the recommendations of WHO for initial 6 month breast feeding have scientific value and advantage in growth, development and protection of child against infections.

CONCLUSION

LAB have great potential of fighting against human pathogens. Consumption of LAB rich dairy products and fermented foods may greatly benefit our digestive and immune system including gut microbiome.

Keeping in view the limitations of *in vitro* studies, it is suggested that further *in vivo* studies are required for clear picture of the role of LABs. Better strains of LAB as probiotics are required to solve the problems related to deficiency of probiotics.

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