

Probiotics as Living Drugs to Resolve Urinary Tract Infections

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Abstract: The increasing consumption of probiotics is due to progress made in understanding the use of probiotics. In the past, probiotics were used to treat UTIs mostly because of an alarming concern regarding rise in antibiotic resistance.

Lactobacillus and *Bifidobacterium* are mostly use on a large scale nowadays. They are gut beneficial bacteria. These organisms have enzymes, H₂O₂, lactic acid, and other acids which are utilized as antimicrobial agents to treat digestive pain, eczema, tumors, and ulceration. Dairy-based products like milk, yogurt, cheese, ice cream, milk powder, dark chocolate, fermented milk etc., mostly contain probiotics.

Samples were collected from different markets. All these samples were enriched for 24 hours following morphology observation by catalase test, gram staining and biochemical test. Antimicrobial activity was checked by measuring zone of inhibition formed by isolated species against uropathogens, namely *E-coli* and *Klebsiella*. A current study showed results that were probable to promote rate of UTI treatment in women who are above 50 years. It was found that natural products contain probiotics which allow the consumer to get their beneficial health effect. These probiotics colonize in the intestine and act as a barrier to prevent urinary tract infections.

Keywords: Probiotics, Urinary Tract Infections, *Lactobacillus*, *Bifidobacterium*, *E.coli*, Colorectal cancer.

INTRODUCTION

There are 2 types of bacteria i.e. good bacteria and the bad ones. Good microorganisms which provide benefits to the human body are most probably called probiotics. Probiotics helps to improve microbial balance in host body. These are very useful as well as human friendly bacteria and are used as microbial treatment [1].

Microbial species with probiotic properties belongs to *Bifidobacterium* and genera *Lactococcus*.

Antimicrobial techniques were seen in *Lactobacillus* which include adhesion inhibitors, organic acids, carbon dioxide, bacteriocins, hydrogen peroxide, and low-molecular weight antimicrobial substances [2]. Antimicrobial activities of these *Lactobacillus* species is mainly relatable with production of organic acids and alternation of Ph [3].

(Madden, J. A. J., & Hunter) investigations have showed that probiotics could stop the threat of eczema in children. Some studies have been conducted on animals which prove that probiotics have anti-tumor property as well as antioxidant property to fight infections. Probiotics have noteworthy functions to reduce digestive pain, helps in sensitive bowel, ulcerative colitis and is useful to avoid allergies [4].

Probiotics have a list of mechanism of action ranging from immunomodulation, short chain fatty acid, bacteriocin production, lowering of gut pH and nutrient competition to stimulate barrier function of mucosa. Probiotics could influence several phases of innate and acquired responses which include: induction of IgA secretion, phagocytosis,

T-cell responses modification, improvement in responses of Th1 and weakening responses of Th2.

The main products are diary-based including fermented milks, cheese, ice cream, , dark chocolates, buttermilk, yogurts, milk powder and curd [5], whereas nondairy food products consist of nutrition bars, variety of juice, cereals and soy based products [6].

URINARY TRACT INFECTION

UTIs commonly begin as infections of bladder (cystitis), but can progress into pyelonephritis i.e. acute kidney infections, and finally causing renal failure and scarring. These infections can be treated by probiotics i.e. non antimicrobial therapeutic approaches, which prevent bacteria from colonizing and inhibiting adherence of bacteria. Tsai CC, *et al.* [2] show that prevention from pathogenic infections caused by *E-coli*, *Salmonella* and *Helicobacter pylori* can be achieved from the use probiotics [7].

Barrons R and Tassone D, have suggested fighting against UTIs by consuming probiotics and fermented milk products [8]. *Lactobacilli* species can act against UTIs as they have several growth effecters including hydrogen peroxide, bacteriocin, biosurfactants and lactic acid [9]. These species can adversely affect fimbrial structure and adhesion that seem to be inhibitory for growth of uropathogenic *E.coli* [10].

PREVENTION OF VAGINITIS

Colonization of pathogenic bacteria including *Gardnerella vaginalis* or *Escherichia coli* is generally accounted as abnormal flora. In women, this abnormal floracan cause symptomatic infections in the vagina [11]. Bacterial vaginitis

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can be easily cured by orally administering *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuterias* mentioned in previous study [12].

CONSTIPATION TREATMENT

Different studies shows that using probiotic strain *L. rhamnosus*, *L. shirota* and *B. infantis*, decrease in abdominal pain and increase in defecation frequency have seen in people with irritable bowel syndrome (IBS) and constipation [13,14].

COLORECTAL CANCER

Western diet is a diet that consists of low fiber or increase consumption of fat and meat. This diet is the main cause of colorectal cancer. It has been observed that this cancer can increase the levels of Clostridium and Bacteroides and can decrease *Bifidobacterium* levels [15]. Reduction in the colorectal cancer actual risk and fecal enzyme activity can be achieved by selective intake of *lactobacilli* [16].

LACTOSE INTOLERANCE

Probiotics can effectively act as immune system enhancer [17]. Milk sugar (lactose) is converted into lactic acid by LAB i.e. lactic acid bacteria that make conditions acidic and inhibit pathogenic growth.

STUDY AIM

To isolate the probiotics from natural products and determine high efficacy of probiotics against uropathogens.

MATERIALS AND METHODOLOGY

For the isolation of probiotics, natural products (yoghurt, butter, infant’s powder milk, chocolate and cheese etc.) were used from different markets samples was collected. Samples include butter, different infant’s powder milk, chocolate, yoghurt, apple, cheese, apple peels etc.

Different samples were used for the isolation of multiple

species of probiotics. 1 gram of sample were added in 5ml MRS broth to enriched the samples for growth enhancement and incubated for 18 to 24 hours at 37°C.

Next day used De Man Rogosa and Sharpe (MRS) agar, streaking were done on plates and incubated for 24 hours at 37°C for the growth of probiotics. Next day after 24 hours colonies were showed on MRS agar and microscopy were done by gram staining. After that some catalase test and sugar tests were applied like lactose, mannitol, sucrose, glucose for the confirmation of microbial specie of probiotics, and then antimicrobial activity of probiotics was checked.

ANTIMICROBIAL ACTIVITY

In this research used molten nutrient agar that was poured into sterilized Petri plates. After agar solidified used spread plate technique on the agar surface to spread the samples of probiotics on agar surface, then well were made on MRS agar which contain samples of probiotics, 0.1ml inoculum were added from MRS broth, that have tested organisms such as (*Klebsiella* specie and *E.coli*). Next day zones were measured in mm from incubated plates.

RESULTS

Using different samples from natural products, we isolated several species of probiotics. Moreover, most of them showed positive zone of inhibition (Table 1).

Table 1. Sugars Utilization Test.

Sugar Name	<i>Enterococcus durans</i>	<i>Streptococcus thermophilus</i>	<i>Lactobacillus bulgaricus</i>	<i>Lactobacillus reuteri</i>
Glucose	+VE	+VE	+VE	+VE
Mannitol	-VE	-VE	-VE	-VE
Sucrose	-VE	-VE	-VE	-VE
Lactose	+VE	+VE	+VE	+VE

Table 2. Zones of Inhibition against *Klebsiella* Species and *E.Coli*.


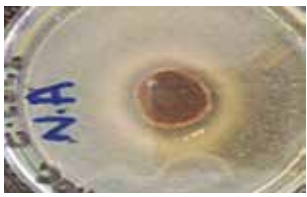
Samples	Tested organism	Isolated organism	Antimicrobial activity
Chocolate	<i>Klebsiella</i> specie	<i>Enterococcus durans</i>	
Chocolate	<i>E.coli</i>	<i>Enterococcus durans</i>	

Table 2 contd.



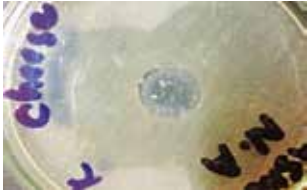
Samples	Tested organism	Isolated organism	Antimicrobial activity
Butter	<i>Klebsiella</i> specie	<i>Enterococcus durans</i>	
Butter	<i>E.coli</i>	<i>Enterococcus durans</i>	
Infant's milk powder	<i>Klebsiella</i> specie	<i>Lactobacillus reuteri</i>	
Infant's milk powder	<i>E.coli</i>	<i>Lactobacillus reuteri</i>	
Infant's milk powder (lactogen)	<i>Klebsiella</i> specie	<i>Lactobacillus reuteri</i>	
Infant's milk powder (lactogen)	<i>E.coli</i>	<i>Lactobacillus reuteri</i>	
Yoghurt	<i>Klebsiella</i> specie	<i>Lactobacillus bulgaricus</i>	

Table 2 contd.

Samples	Tested organism	Isolated organism	Antimicrobial activity
Yoghurt	<i>E.coli</i>	<i>Lactobacillus bulgaricus</i>	
Cheese	<i>Klebsiella specie</i>	<i>Streptococcus thermophilus</i>	
Cheese	<i>E.coli</i>	<i>Streptococcus thermophilus</i>	

ANTIMICROBIAL ACTIVITY

Agar well diffusion technique was performed to check probiotic antimicrobial activity against *E. coli* and *klebsiella* i.e. uropathogenic organisms. Sensitive zone was observed which is an indication of probiotic showed antimicrobial activity against pathogenic bacteria (Table 2).

DISCUSSION

In the current study, different probiotic species were isolated from collected samples which include: chocolates, butter, dairy product, different infant's milk powder etc. Gram staining tests were performed on the isolated species which resulted in identification of *Coccobacilli* and Gram positive *bacilli*. Several biochemical tests were also performed for the confirmation of genus and species. From infant's milk powders, *Lactobacillus reuteri* was isolated. It was found to be gram positive and with thick rods (Fig. 1). Bacterial vaginitis can be easily cured by orally administering *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* [18].

From the cheese samples, *Streptococcus thermophiles* was isolated. Through catalase test which was negative, it was confirmed that isolate species were of *Streptococcus thermophilus*. Its morphology was found to be cocci in bunches diplococcic and tetrads (Fig. 2). *Streptococcus thermophiles* plays a vital role in food industry and are mostly required in the making of dairy products

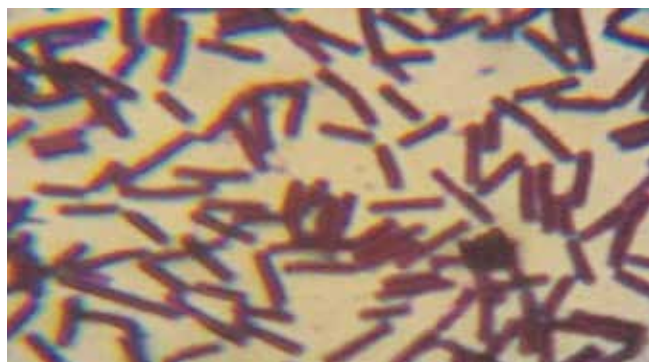


Fig. (1). From Infant's Powder Milk, Isolated *Lactobacillus reuteri*.

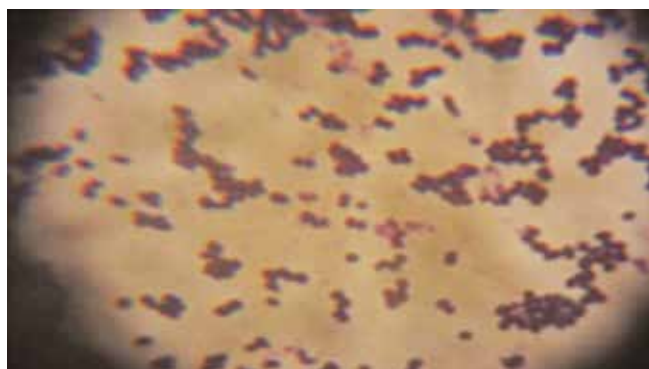


Fig. (2). From Samples of Cheese, Isolated *Streptococcus thermophiles*.

From yoghurt samples, *Lactobacillus bulgaricus* was isolated. It was found to be Gram positive bacteria with positive catalase test. Its morphology consisted of highly long rods (Fig. 3).



Fig. (3). From Samples of Yoghurt, Isolated *Lactobacillus bulgaricus*.

From few chocolate and butter samples, *Enterococcus durans* was isolated. It showed negative result in catalase test and positive results for glucose and lactose sugar. It was found to be gram positive bacteria. Its morphology consists of ovoid shape, short chains and grouped in pairs (Fig. 4).

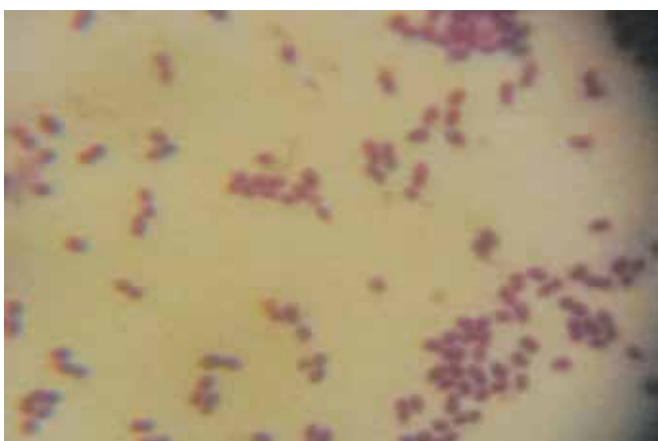


Fig. (4). From Samples of Butter and Chocolate, Isolated *Enterococcus durans*.

To check the antimicrobial activity of the species isolated from samples, agar well diffusion technique was performed. *Klebsiella* species and *E.coli* were the uropathogens against which zone of inhibition was measured. Against *E.coli*, antimicrobial activity was observed by the cultures of *Lactobacillus reuteri*, *Enterococcus durans*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Fig. 5). The measured zone of inhibition on the agar plate of these cultures was found to be 30mM, 24mM, 21mM and 21mM respectively (Table 3).

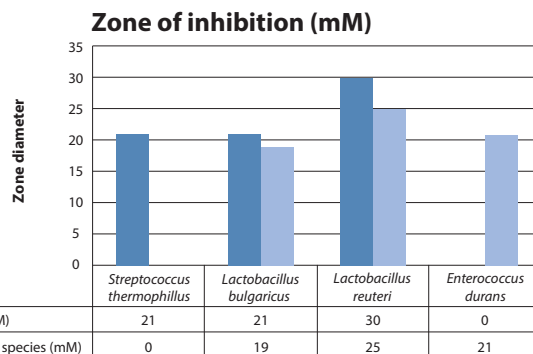


Fig. (5). Measurements of Zones of Inhibition against Enteric Bacteria in Graph.

Table 3. Measured Zone of Inhibition of Isolated Probiotics Species against Enteric Bacteria.

S. no.	Test organisms	Name of isolates	Zone of inhibition (mM)
1	<i>E.coli</i>	<i>Lactobacillus reuteri</i>	30mM
		<i>Enterococcus durans</i>	24mM
		<i>Lactobacillus bulgaricus</i>	21mM
		<i>Streptococcus thermophilus</i>	21mM
2	<i>Klebsiella</i> species	<i>Lactobacillus reuteri</i>	25 mM
		<i>Enterococcus durans</i>	21mM
		<i>Lactobacillus bulgaricus</i>	19mM
		<i>Streptococcus thermophilus</i>	0mM

Against *klebsiella* species, antimicrobial activity was observed by the cultures of *Lactobacillus reuteri*, *Enterococcus durans*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus*. The measured zone of inhibition on the agar plate of these cultures was found to be 25mM, 21mM, 19mM and 0mM respectively (Table 3).

Through this study, beneficial health effects of dairy and natural food products can be seen in the protection of urinary tract infections. Menopausal women are at most risk for developing UTIs and are commonly seen in this age group; hence they can attain more benefit from natural and dairy products as they help in recovery from UTIs and provide protection against pathogens causing urinary tract infections. In an emergency we can use these natural products to start the treatment of infection at home.

CONCLUSION

In menopausal women, there is an increased risk of UTIs, due to several factors. Low vaginal pH causes UTIs by promoting survival of different pathogenic bacteria. In the current study, it is concluded that we can use home product like chocolates, infant’s milk powder, cheese etc. to start the treatment of urinary tract infection at home and immediately which is also helpful to halt the increase infection. We isolated different species of probiotics which showed positive antimicrobial

activity. This conclusion promotes the use of probiotics in the women who are 50 years and above for beneficial health effects. These probiotics, like lactic acid bacteria, can be found in natural products and provide protective effect by colonizing in the gut system and act as a barrier against uropathogens. They can also be used to treat UTIs.

CONFLICT OF INTEREST

Declared none.

ACKNOWLEDGEMENTS

Declared none.

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