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A Comparative Study on Probiotic Organisms Isolated from Different food and Milk Products and Medicinal Capsules.

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Research Article

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ABSTRACT

A number of health benefits have been claimed for probiotic bacteria such as Lactobacillus species and Enterococcus species. Beneficial effects of food, milk product and medicinal capsules with live microbes (probiotics) on human health are being increasingly promoted by professionals. These benefits include antimicrobial, anti-mutagenic effects, anti-carcinogenic properties, improvement in lactose metabolism, reduction in serum cholesterol and immune system stimulation. Several studies in recent years have shown the benefits deriving from the ingestion of probiotics. The objective of this study was to screen ten potential probiotic organisms from total of 10 different food samples, milks products, and medicinal capsules were collected. The organisms identified on the basis of phenotypic characters and biochemical properties were *Enterococci durans, Lactobacillus rhamnous, Lactobacillus plantarum, Lactobacillus paracasei, and Lactobacillus lactis.* It was observed that similar types of probiotic organisms were isolated from food items, milk products and medicinal capsules.

INTRODUCTION

Probiotics represent probably the archetypal functional food, and are defined as alive microbial supplement, which beneficially affect the host by improving its intestinal microbial balance. Kollath in 1953, first defined the term “probiotic”, when he suggested the term to denote all organic and inorganic food complexes as “probiotics,” in contrast to harmful antibiotics, for the purpose of upgrading such food complexes as supplements. Vergio, in his publication “Anti- und Probiotika”, compared the detrimental effects of antibiotics and other antimicrobial substances with favorable factors (“Probiotika”) on the gut microbiology. Lilly and Stillwell proposed probiotics to be “microorganisms promoting the growth of other microorganisms”. The term probiotic was technically defined by an Expert Committee as ‘‘live microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition’’. This means that the microorganisms must be alive and present in high numbers, generally more than 109 cells per daily ingested dose. Each product should indicate the minimum daily amount required for it to confer specific health benefit(s) [1].

Early scientific studies on microorganisms, during the second part of the 19th century which dealt with the interactions with the human host, suggested their benefits for digestion and the beneficial association of vaginal bacteria by production of lactic acid from sugars, thereby preventing or inhibiting the growth of pathogenic bacteria. These findings and other information on the early stages of development toward biotherapeutic concepts and the utilization of functional major metabolic product were generally grouped as “lactic acid bacteria” (LAB) [2]. Recent research has underlined the importance of a vital and “healthy” microbial population of the gastro intestinal tract (GIT). Increased research efforts during the last

three decades, have confirmed the beneficial association of LAB with the human host. Metschnikoff suggested the longevity of the Caucasians to be related to the high intake of fermented milk, and considered that lactic acid production, resulting from sugar fermentation by LAB, to be particularly beneficial. The Bifidobacteria, another group producing lactic acid, phylogenetically distant but commonly accepted to form part of the LAB, were discovered in 1889 and described in the early 1900s by Tissier to be associated with the feces especially of breast-fed infants. When compared to formula-fed infants, a lower incidence of intestinal upsets was observed for infants receiving mother's milk. Thereby the assumption was made about the beneficial association of Bifidobacteria with the human GIT.

Functionality of probiotics

The beneficial effects of food with added live microbes (probiotics) on human health, and in particular on children and other high-risk populations, are being increasingly promoted by health professionals. It has been reported that probiotics can play an important role in immunological, digestive and respiratory functions and could have a significant effect in alleviating infectious disease in children. However, some health benefits, e.g., immune modulation, may be achieved even with dead bacteria [3].

Prevention of diarrhea

Several probiotic strains, especially *Lactobacillus rhamnosus* have been shown to prevent or alleviate infantile diarrhea, caused mainly by rotavirus. It is also well-established that some probiotic strains can both prevent and shorten antibiotic-associated disorders [4]

Stimulation of the immune system

Many human studies performed to investigate the effects of probiotic cultures on the immune system reveal that probiotic bacteria are able to enhance both innate and acquired immunity by increasing natural killer cell activity and phagocytosis, changing cytokine profiles, and increasing levels of immunoglobulin's. Two probiotic strains have been developed with a particular focus on their enhancing effects on immune responses*: Bifidobacterium lactis* and *Lactobacillus rhamnosus*. Both strains have been demonstrated in several studies to enhance natural immune function in healthy people [4].

Inflammatory bowel disease

There is growing evidence that probiotics have a potential therapeutic benefit for patients suffering from IBD. Controlled clinical studies have demonstrated that probiotics are efficacious in the maintenance of remission of pouchitis, prophylaxis of pouchitis after the formation of an ileoanal reservoir, maintenance of remission of ulcerative colitis, and treatment of Crohn's disease [5].

Lactose intolerance

Bacterial cultures, yogurt starter cultures as well as some probiotic cultures are known to improve the lactose digestion in lactose maldigestors. Subjects suffering from lactose intolerance have a very low concentration of the lactose-cleaving enzyme β-galactosidase, and bacteria in fermented or unfermented food products release their β-galactosidase in the small intestine, where it supports lactose digestion [6].

Allergies

Pelto et al. found that *Lactobacillus rhamnosus GG* confers an immunostimulatory effect in healthy adults. Probiotics have also been used successfully in the management of atopic eczema in infants. Furthermore, *Lactobacillus rhamnosus GG* proved to be effective in the prevention of early atopic disease in children at high risk. The *Lactobacillus rhamnosus GG* product when given prenatally to mothers and postnatally for 6 months to the mothers or to their infants directly, reduced the frequency of atopic eczema in the probiotic group to half that of the placebo group at the age of 2 years. The preventive effect was reconfirmed at the age of 4 years [7]

Cancer

A few epidemiological studies indicate that consumption of fermented dairy products containing *Lactobacilli* or *Bifidobacteria* lowers the incidence of colorectal cancer. However, there is some indirect

evidence based on several markers applied in human studies (e.g., fecal enzyme activities, fecal mutagenicity and genotoxicity, immunological markers), which shows that probiotics reduce the risk of 11colon cancer. A case-control study conducted in Japan with 180 cases and 445 controls revealed that habitual intake of lactic acid bacteria reduces the risk of bladder cancer [8].

Respiratory tract infections

Respiratory effects of probiotics in animal models have included attenuating allergic airway responses and protecting against respiratory pathogens. Dendritic cells appear central to directing the beneficial immune response to probiotic bacteria and in translating microbial signals from the innate to the adaptive immune system, whereas regulatory T cells are emerging as potentially key effectors of probiotic-mediated responses, particularly in the reduction of allergic inflammation. Despite progress in basic research, clinical trials of probiotics in allergy/asthma and respiratory infection have been highly variable at best, leading to an undermining of confidence in this potential therapeutic strategy [9].

Constipation

Studies carried out on the effects of *lactic acid bacteria* on constipation and intestinal motility, have shown reduced severity of constipation and an improved bowel movement frequency and stool consistency in constipated but otherwise healthy people after consumption of a fermented milk drink containing *Lactobacillus casei strain Shirota*. Administration of *Bifidobacterium longum BB536* to constipated women resulted in a significantly increased defecation frequency and stool softness. A positive influence of *Bifidobacterium longum BB536* on the "regularity" was also reported for elderly people [10].

Urogenital tract infections

Studies suggest that probiotic preparations given orally or intravaginally may provide a therapeutic source of *Lactobacilli* to help control urogenital infections in women. A case-control study with 139 females with acute urinary tract infection and 185 controls revealed that consumption of fermented milk products containing probiotic bacteria was associated with a decreased risk of recurrence of urinary tract infection[11]

Helicobacter pylori infection

Several probiotic strains have been shown to inhibit Helicobacter pylori in vitro, which has been associated with gastritis, stomach carcinoma, gastric ulcer, and lymphomas. Human studies confirmed this inhibitory effect on Helicobacter pylori, which seems to be independent of the viability of the bacteria [12].

High cholesterol

Many human studies have evaluated the effects of culture-containing dairy products or probiotic bacteria on cholesterol levels with equivocal results. A fermented milk containing Enterococcus faecium and Streptococcus thermophilus was reported to produce a small but significant decrease in total and LDL-cholesterol in patients with primary hypercholesterolemia [13].

MATERIALS AND METHODS

Sample collection

A total of 10 different samples were collected and were carried to the laboratory in plastic containers for achievement of the isolation procedure. The samples included curd, cheese, butter, yoghurt, yakult, idly batter, darolac (capsule), bifilac (capsule), and vibact (capsule).

Isolation of the organisms

Samples such as curd, butter, yogurt, cheese, and yakult and idly batter were directly inoculated with the sterile inoculating wire loop on the MRS agar taken and streaked on to the MRS [de Man, Rogosa and Sharpe] agar and the samples such as Dark chocolate (born villa), vibact, bifilac, and darolac were processed to single fold dilution and 10^{-4} dilution was taken for inoculation onto MRS agar .The inoculated plates were incubated at 37°C for 18-24 hours. After incubation, well isolated colonies were observed.

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Morphological identification

The bacteria were Gram stained and observed under light microscope [14] (100×)

Biochemical test

Biochemical properties of the isolates were tested according to Bergey's Manual of Systematic Bacteriology. The sugars used were glucose, lactose, xylose, sucrose, arabinose, maltose, ribose and mannitol to identify the isolates.

Effect of temperature on the growth of the organism

Procedure

10ml of MRS broth was taken and the sample was inoculated. The broth was incubated at different temperatures like 10°C, 15°C, 37°C and 45°C at 24-48 hours respectively and the growth was monitored.

Respirometric effect

Aerobic micro-organisms respire utilizing the oxygen and releasing, CO_2 rate of respiration is directly proportional to size of microbial population and their activity. CO_2 released during respiration is estimated which gives an indirect evidence of microbial activity .The CO_2 liberated is absorbed by sodium hydroxide. The CO_2 thus produced is estimated by titrating sodium hydroxide against HCl.

Amount of CO_2 liberated = difference in titer value×normality of acid× volume of NaOH× equivalent weight of CO_2 / Size of sample

Antimicrobial activity

Agar Well Diffusion Technique

Molten nutrient agar was poured into sterilized Petri plates and allowed to stand. 0.1ml of culture was inoculated on the agar surface by spread plate technique. Wells were made in the solidified agar. 0.1ml of the inoculum from different organisms maintained as pure cultures in MRS broth was taken in the wells. The plates were incubated at 37°C for 24 – 48 hours and the results were analyzed.

RESULTS AND DISCUSSION

Probiotics are organisms such as bacteria or yeast that are believed to improve health. They are available in supplements and foods. Researchers believe that some digestive disorders happen when the balance of friendly bacteria in the intestines becomes disturbed. This can happen after an infection or after taking antibiotics. Intestinal problems can also arise when the lining of the intestines is damaged. Taking probiotics may help.The samples taken for isolation of probiotic organisms were curd, cheese, butter, yoghurt, yakult, idly batter, darolac (capsule), bifilac (capsule), vibact (capsule). 0.1ml of the samples was inoculated on MRS agar for the isolation of microorganisms Morphological identification of the organisms isolated on MRS agar was carried on by Gram staining and it was observed that Gram positive bacilli and Coccobacilli were identified from all the samples except dark chocolate . Gram negative cocci were isolated from dark chocolate. Further identification of the organisms was carried on based on the biochemical tests (carbohydrate fermentation test). The morphological as well as the biochemical studies reveal the presence of *Enterococcus durans* in curd , *Lactobacillus rhamnosus in* cheese:, *Lactobacillus paracasei* in yogurt: *Lactobacillus lactis* in butter,*Lactobacillus plantarum in* idly batter, *Lactobacillus paracasei in* yakult, *Enterococcus durans* in dark chocolate*, Lactobacillus rhamnosus* in darolac*, Lactobacillus plantarum in* bifilac and *Lactobacillus paracasei* in vibact(Table 1) [15]*.* The microbial load present in the samples was analyzed by Respirometry method. It is an indirect method of estimating the quantity of microorganisms present in the sample which determines the quantity based on the amount of CO2 evolved which is equal to the number of organisms present .The samples which showed the maximum probiotic organisms load were yakult, idly batter, bifilac, darolac and vibact .This result indicates that maximum beneficial organisms are present in these samples which are useful in improving human health (Table 2). The antimicrobial activity of the samples was also analyzed to

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determine their potency as antimicrobials against common organisms like E.coli, Pseudomonas and Staphylococcus. It was observed that curd, idly batter and bifilac showed an inhibition zone diameter of 1cm,0.5cm and 0.2cm against E.coli ;2cm,1.6cm and 0.9cm against *Pseudomonas* and 1.2cm,2.3cm 0.2cm against *Staphylococcus* respectively (Table 3).

Table 1: Morphological identification and sugar utilization tests

Samples test	Curd	Cheese	Yogurt	Butter	Yakult	Idly	Dark chocolate	Darolac	Vibact	Bifilac
Gram character	+	+	+	+	+	+	_	+	+	+
Morphology	Cocco bacilli	Rods	Rods	Coccobacilli	Rods	Rods	Cocci	Rods	Rods	Rods
Sugar utilization										
Glucose	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+
Xylose	_	_	_	+	_	+	_	_	_	+
Sucrose	_	_	+	+	+	+	_	_	+	+
Arabinose	_	_	_	+	_	+	_	_	_	+
Maltose	+	+	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	_	+	+	+
Temperature										
10°C	_	_	_	_	_	_	_	_	_	_
15°C	+	+	+	+	+	+	+/_	+	+	+
37°C	+	+	+	+	+	+	+	+	+	+
45°C	+	+	+	+	+	+	+	+	+	+
Organisms identified	Ec. durans	Lb. rhamnosus	Lb. paracasei	Lb. rhamnosus	Lb. paracasei	Lb. plantarum	Ec. durans	Lb. rhamnosus	Lb.paracasei	Lb. Plantarum

Table 2: Respirometry method

Sr .No.	Samples	Titer Value	Amt. Of CO2 Evolved
1	curd	7.5ml	1.65
2	cheese	12.5ml	2.75
3	yoghurt	4.5ml	0.99
4	butter	6ml	1.32
5	idly batter	18.5ml	4.07
6	dark chocolate	5.5ml	1.21
7	yakult	23.6ml	5.192
8	bifilac	21ml	4.62
9	darolac	20ml	4.4
10	vibact	19.5ml	4.29

Table 3: Antimicrobial Activity

S.no.	Test organisms	Test samples	Zone of inhibition (in cm)
1	*E.Coli*	Curd	1cm
		Idly batter	0.5cm
		bifilac	0.2cm
2	*Pseudomonas*	Curd	2cm
		Idly batter	1.6cm
		bifilac	0.9cm
3	*Staphylococcus*	Curd	1.2cm
		Idly batter	2.3cm
		bifilac	0.2cm

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CONCLUSION

From the above studies we conclude that the organisms isolated and identified as probiotic organisms such as *Enterococcus durans, Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus paracasei,* and *Lactobacillus lactis* from various food products. *Lb. rhamnosus, Lb.plantarum* and *Lb.paracasei* were the organisms which were isolated from the medicinal capsules. The organisms isolated were similar from both food products as well as medicinal capsules. So it is always better to supplement the human body with useful organisms through consumption of food products rather than through medicines. The food products like idly batter and curd showed beneficial organisms and antimicrobial activity also which enables good health and also improves immune status.Further study is required to determine their antimicrobial activity against variety of organisms.

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