

Study of Normal and Pathogenic Bacteria in Medicinal Probiotics

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Abstract

There are many medicinal probiotics available which are usually bacteria but sometimes also yeasts. These medicinal probiotics, sometimes contain some undesired products like pathogenic aerobic or anaerobic bacteria. These are hitherto unexplored areas of public health research. Hence here we have tried to study the presence of these types of pathogenic bacteria. Some pathogens were found in the medicinal probiotics.

Keywords: probiotics; disease

Introduction

Probiotics is a Greek word which means 'for life'. The term probiotics refers to non-pathogenic live microorganisms in adequate doses which can exert favorable function on the host microbiota and health. Live microorganisms that help to treat various pathologies including irritable bowel syndrome (IBS), Inflammatory Bowel Disease (IBD) (Ulcerative colitis and Crohn's disease), Constipation, Diarrhoea (acute and antibiotic related). It also helps to treat in Atopic eczema [1]. Probiotics medicines or food items like (curd, yogurt, kefir, tempeh, sauerkraut, cheese) are also helpful in treating and/or controlling Non-Communicable Disease (NCD) such as Diabetes Mellitus (DM), Hypertension (HTN). Probiotics are well known for developing effects on the changes of gut microbial flora. Since oral cavity forms the first part of GIT (Gastro Intestinal Tract), it can be quite possible for probiotics to influence the oral microbial flora as well. Probiotic bacteria can be formulated as pharmaceutical products or as dietary supplements and also beneficial for treating necrotizing enterocolitis, liver encephalopathy and bacterial vaginosis [2]. Nowadays medicinal probiotics are used extensively to avoid or eliminated antibiotics resistance and medicinal probiotics are also much safer than food items of probiotics.

Role of Medicinal Probiotics in Different Diseases

Probiotics are live microorganisms which impart some health benefits to the host when taken in small amounts. There are many commercial probiotics contain ingredients like such live microorganisms. However, till now there is no uniform rule regarding their usage and no study has assessed the presence of pathogens in these probiotics. They can be used both for gut ailments as well as for other conditions.

The applications of medicinal probiotics are as follows

Headache-The definition of psychobiotics should be expanded to any extrinsic influence whose effect on the brain is bacterially-mediated. Psychobiotics are beneficial bacteria (probiotics) or support for such bacteria (prebiotics) that control interconnection between bacteria and brain. Psychobiotics can changes in emotional, cognitive, systemic and neural indices, which exert anxiolytic and antidepressant effects in brain. Interconnection between bacteria-brain which psychobiotics exert effects include the enteric nervous system and the immune system [3]. According to current research, probiotics are capable of resolving stress and reducing cortisol levels in humans, that

ultimately points to of an additional mechanism by which they can influence migraine headaches [4].

Recently, probiotics have been used for treating migraines contemplating or considering that the multiple mechanisms by which gastro-intestinal (GI) microorganisms may affect the function of the central nervous system (CNS). The initial motive of migraine treatments includes relieving pain, restoring function, and reducing headache frequency with current treatments for migraine centering on decreasing the frequency, severity, and duration of migraine attacks [5,6,7].

Irritable Bowel Syndrome - irritable bowel syndrome (IBS) is a functional gastrointestinal disease (FGID) that has no accurate definition of aetiology. IBS is characterized by chronic abdominal pain and altered abdominal practice, like constipation, diarrhoea, or alternating constipation and diarrhoea, which are further integrate with abdominal bloating, but this complication is not relevant for every case of IBS [8]. The pathophysiology of IBS indicates gastrointestinal (GI) motility dysfunctions, bile acid malabsorption (BAM), conversion of gut microbiota with pathogenic microorganisms and alterations in enteric nervous system (gastrointestinal tract is the only internal organ to have evolved with its own independent nervous system known as enteric nervous system). In some research it has been observed that there is an extreme/strong correlation between IBS and chronic micro-inflammation or low-grade inflammation at the intestinal mucosa epithelial surfaces that persuade modifications in the natural phenomenon of gastrointestinal tract GIT [9,10,11].

The mechanisms of action of probiotics in the gastrointestinal tract and incorporate gut microbiota regulation by promotion and inhibition of adherence of pathogens to the gut epithelia by the formation of short chain fatty acids (SCFAs), bacteriocins, and biosurfactants which can improve in the gut barrier function of the gut mucosa by downregulation of low-level mucosal immune activation, increasing the mucus layer, and production of protein; regulate the inflammatory mediators like cytokines (immunomodulating agents), by stimulate secretory immunoglobulins (IgA) production resulting improvement of the gut immunity and intensification of gut-brain communication [12, 13]. The mechanisms of probiotics involved in the regulation of gut microbiota depends on the capability of the probiotic strains or combinations of probiotic strains to inhibit, replace, or interfere/impede in the process of

adherence of pathogenic strains [14, 15, 16]. Genus, species and strain variability play a principal role in considering the level of adhesion and adherence competing properties of the probiotics. Many research showed that the capability of specific probiotic strains to replace and competitively inhibit the adherence of pathogenic strains like *Salmonella*, *Staphylococcus aureus*, *Escherichia coli*, *Clostridium difficile*, *Staphylococcus aureus*, and *Bacteroides vulgatus*, *Listeria monocytogenes* [17]. The formation of different antimicrobial substances like short chain fatty acids (SCFAs), bacteriocins and biosurfactants, and another principal mechanism of probiotics are producing de-conjugated bile acid which further involved in modulation of gut microbiota. SCFAs such as (butyric, propionic, lactic, and acetic acids) are a part of the amalgam derive after the metabolism of carbohydrates (CHO) by probiotic bacteria and they also lowering the overall pH of the small intestine, for hampering the growth of pathogenic bacteria [18, 19, 20].

Inflammatory Bowel Disease

This term includes two different clinical states: Crohn's disease (CD) and Ulcerative colitis (UC). Crohn's disease can affect any parts of the gastrointestinal tract (GIT), from the oral cavity to the anus. Commonly shared symptoms for CD and UC are diarrhoea also with bleeding (dysentery), obstruction, abdominal cramps and/or pain, weight loss, fever, weakness, fatigue, loss of appetite and malnutrition. In the course of IBD, extra manifestation sometimes can also be observed and more interestingly, uncharacteristic symptoms contribute to an earlier identification of the disease in patients with early changes within the digestive tract [21]. The intestinal microflora, also called the microbiome, has many functions in our health. Its primary function is to maintain the normal digestive system. In addition, intestinal bacteria produce vitamins like (B2, B3, B9), communicate with the intestinal epithelium and modulate the host's action and also stimulate the immune system [22,23]. The microorganisms with positive effects can be used to improve health. Microorganisms that, after appropriate preparation and administration, have a positive effect on the body are called probiotics.

Atopic Dermatitis: Atopic dermatitis or eczema is the commonest dermatologic problems, especially in children. Several studies have hypothesized that alteration of gut-colonizing microbes might have

induced and conditioned the development of the disease. Thus, modulation of microbial diversity and abundance might help alleviate symptoms and conditions for patients. Given the ability of commensal and symbiotic microorganisms in modulating the immune system, probiotics administration has been studied in previous research in the management of eczema. However, until today, there are conflicting results between studies making inconclusive recommendations towards probiotics supplementation in the management of atopic dermatitis. This case-based review was done to assess and evaluate the therapeutic efficacy of probiotics supplementation in the management of eczema in children [24].

Medicinal Probiotics

Probiotic medicines are contemplated as health foods, but are however not regulated by the Food and Drug Administration (FDA) as a drug.

- *Lactobacillus* spp. - This may be the most common probiotic. It is mainly found in curd, yogurt and other fermented foods. Different strains can help with diarrhoea, Functional abdominal pain, *Helicobacter pylori* infection, Hepatic encephalopathy, Irritable bowel syndrome, NAFLD/NASH and may help people who cannot digest lactose, the sugar in milk.
- *Bifidobacterium* spp- Mainly present in dairy products. It may help ease the symptoms of irritable bowel syndrome (IBS) and some other conditions.
- *Saccharomyces boulardii* - is a yeast extensively found in probiotics. It appears to help acute infection, diarrhoea, Antibiotic-associated diarrhoea, *C. difficile*-associated diarrhoea, *C. difficile* infection, Crohn disease and other digestive related problems.

Bacterial strain based probiotic medicines may sometimes cause antibiotics resistance in body whereas yeast based probiotic medicines does not have that risk to develop antibiotics resistance in body so, yeast-based probiotics are much safer than bacterial based.

There is also some research that shows they are useful for problems in other parts of the body.

For example: Oral health, Urinary and vaginal health

Sometimes in some cases, mild side effects might include an upset stomach, flatulence, and bloating, and also diarrhoea for the first couple of days after start taking them. They may also trigger allergic reactions. In acute pancreatitis, probiotics medicine is strictly restricted.

Pattern of bacteria found in different commercial probiotics: Aerobic bacterial probiotics are more commonly present in medicinal probiotic compositions, like *Lactobacillus reuteri* (Lactic acid bacillus), *Lactobacillus casei*, *Streptococcus fecalis/Enterococcus fecalis*, *Streptococcus thermophilus*, *Bacillus mesentericus*, *Lactobacillus acidophilus*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Lactobacillus delbrueckii*, sub spp. *bulgaricus*, *Streptococcus faecalis*, *Bacillus mesentericus*, *Lactobacillus acidophilus*, *Bacillus clausii* spores, *Lactobacillus fermentum*, *Lactobacillus salivarius*, *Lactobacillus reuteri* followed by anaerobic bacteria like *Clostridium butyricum* and yeasts like *Saccharomyces boulardii*.

Materials And Methods

Time of study: The study was carried out from September 2022 to January 2023.

Place of study: Department of Microbiology, BN campus, All India Institute of Hygiene and Public Health, Kolkata.

Type of study: Laboratory based observational study.

Sample size: One hundred and two probiotic samples (102) were tested by us, out of which 52 were curd samples. This sample size was calculated by method of convenience. Here only data of the 52 curd samples are shown. There were 35 loose curd samples bought from sweet shops, 14 packed branded curd from shops, and 3 in-house made yogurts/curd. Fifty (50) samples were medicinal probiotics bought from the market.

Methodology proper

Samples were collected from (A) Kolkata and Bidhannagar, **(B)** Durgapur, West Bardhaman district, West Bengal.

The areas of sample collection have been shown in figure 1.

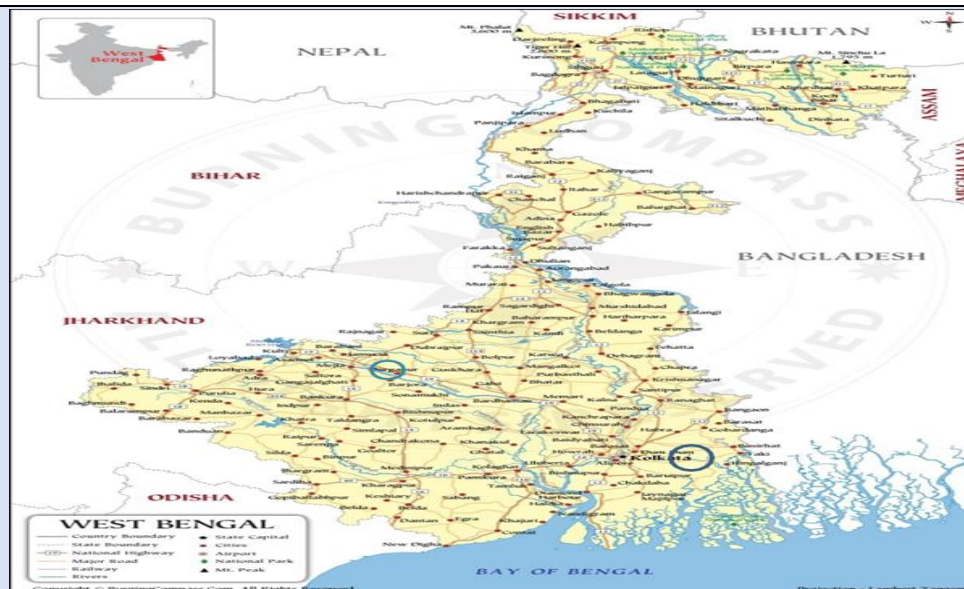


Figure 1: Map showing areas of commercial/medicinal probiotic sample collection in West Bengal in encircled shapes. Durgapur: Kolkata and Bidhannagar

Samples were collected in sterile universal container or if packed, then in packet or in case of curd from sweet shop, earthen containers. In-house samples were made in sterile universal containers. The date of packaging, expiry date and brand name were all noted. Then samples were transported to laboratory in ice-pack or within 4 hours of collection. The pH of the samples was also noted. Then the samples were processed for bacteria and yeasts. Samples were weighed, and specific weight or volume were inoculated on the following media:

- Mac Conkey agar with neutral red as pH indicator (Peptone, Neutral red, agar agar, Lactose, Sodium taurocholate, deionized water) for bacteria.
- Sabouraud's dextrose agar plate (pH 5.6-6) (glucose - 2gm, Peptone 2 grams, Agar agar 2 grams, deionized water 100 ml) for fungi.
- Robertson's cooked meat medium (RCM) for culturing anaerobes, made as per manufacturer's instructions.
- Tomato juice agar for lactobacilli (formula appended below).
- For lactobacilli, plates were kept in microaerophilic incubation using glass candle jar and white burning candle.

Simultaneously, Gram's stain, Albert's stain and conventional Ziehl-Neelson's stain were done from samples directly to detect bacteria, bacteria with metachromatic granules and acid-fast bacilli, respectively.

After inoculating the samples, plates were incubated at 37 Degree C overnight, i.e., for 12 to 16 hours, and

then observed for colonies. Tomato juice agar was incubated in microaerophilic conditions in candle jar. RCM tubes were observed after 48 hours of incubation and Gram stain were done from RCM to detect spore bearing anaerobic bacilli. Colonies on MacConkey agar and Tomato juice agar were identified by colony morphology, Gram stain, Albert's stain for bacteria or yeasts with metachromatic granules and also ZN (Ziehl-Neelson) stain if required. Lactobacilli were identified by Gram stain morphology (Gram positive uniformly staining bacilli) and small punctuate or pitted colonies, or sometimes translucent colonies on Tomato juice agar, with inherent resistance against Vancomycin and variable resistance against Nitrofurantoin. Biochemical tests like motility, Citrate utilization, acid and H₂S on TSI agar were also done from colonies of Gram-negative bacteria, and from Gram positive colonies, standard biochemicals like Catalase (with 3% H₂O₂), coagulase and oxidase were done. Lactococcus was identified by magenta colonies on MacConkey agar and no growth at 44 degree C. Enterococcus spp. were identified by magenta colonies on MAC Conkey Agar, positive growth in 6.5 % NaCl, positive aesculin hydrolysis and positive growth at 44 degree C. Bacillus spp. and E. coli were identified by standard biochemical tests like lecithinase production, indole production, mannitol breakdown and molds by microscopic morphology on Lactophenol cotton blue mount from colonies on SDA (Sabouraud's dextrose agar).

Yeasts were identified by Gram stain, Lactophenol cotton blue mount, Germ tube test, growth on SDA at

44 Degree C (for confirming *Candida albicans*) and also sugar fermentation tests.

For bacteria, their count per ml of sample were calculated.

Hence the data were collected and analyzed. The prevalence of bacteria and yeasts in probiotics were assessed. Recipe of tomato juice agar is given in table 2 below.

Table 2: Formula of modified tomato juice agar.

Ingredients	Amounts
Tomato juice concentrate	2 gm
Peptone	1 gm
Milk powder	0.5gm
Whey powder	0.5gm
Agar agar	1.3 gm

pH of tomato juice agar: 5.0

Results

Mostly the medicinal probiotics contained the bacteria that were written on their contents. However, they also had sometimes some unwanted or unspecified or pathogenic bacteria. These things are important from public health viewpoint. These things should be explored by other workers also. This may be due to probiotic bacteria which can exist in a viable but non-culturable state (VBNC).

Medicinal probiotics from shops grew and contained bacteria, like *Enterococcus* spp. *Lactococcus* spp. *Bacillus* spp., *Enterococcus fecalis*, *Lactobacillus casei*,

Lactobacillus plantarum, *Corynebacterium* spp., *Acinetobacter baumannii* (not mentioned in their contents but present in some) and fungi like *Saccharomyces boulardii*. Some unwanted bacteria like *Acinetobacter baumannii*, *Corynebacterium* were also found in medicines. In medicinal probiotics it has been observed that the mentioned composition is not always true. Some unidentified yeast was also found. According to the findings of the results 4% medicinal probiotics contained pathogenic microorganisms. Results have been illustrated in figure 2. Also, the image of a medicinal preparation is given in figure 3.

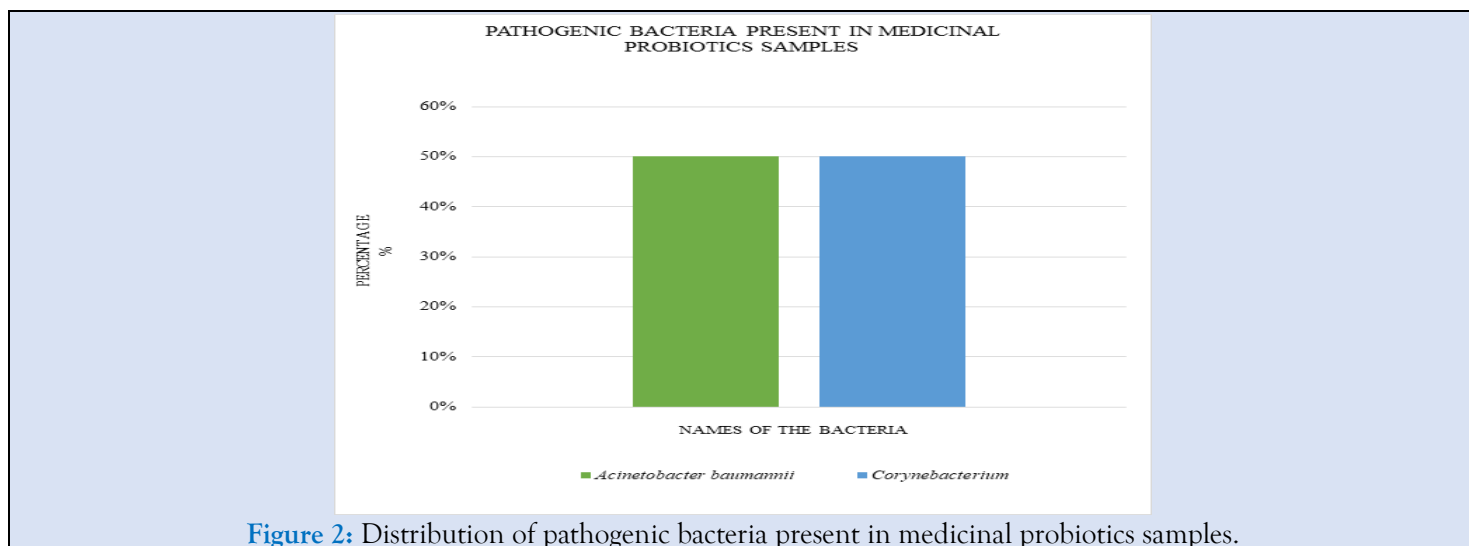


Figure 2: Distribution of pathogenic bacteria present in medicinal probiotics samples.



Figure 3: Medicinal preparation of probiotic

Live probiotic bacteria can exist in a viable but non-culturable state (VBNC). This is why some samples did not grow lactobacilli but showed Gram positive bacilli on Gram stain. No anaerobic bacteria were found in medicinal probiotics. Cell culture estimates numbers of replicating strains but not those in the VBNC (viable but not culturable) state. These can be estimated in further studies.

Discussion

Nobody could feel any problems or symptoms in gastrointestinal tract after consuming medicinal probiotics samples. A total of 12 people consumed the medicinal probiotics, out of which nobody reported any adverse gastrointestinal symptoms. Hence at the of the study we were able to assess the burden of normal as well as pathogenic bacteria and fungi in the probiotic preparations. We found that it is not always safe to consume curd from the market as loose curd can contain many harmful bacteria like *Escherichia coli* and even moulds. This type of study has not been done previously in this part of the country. Hence these findings are very important from the public health view point. Probiotics are now being used for treatment of other conditions like insomnia and dental diseases also. Also, many probiotics can contain more than one type of microorganisms. Our study was also helpful for finding that. Also, we found out a new medium, modified Tomato juice agar which successfully grew Lactobacilli. More such studies are needed in the interesting field encompassing microbiology, nutrition science and public health. Probiotic benefits that are associated with one species or strain do not necessarily hold true for others. The strongest evidence for the clinical effectiveness of probiotics has been in the treatment of acute diarrhoea, most commonly due to

Rotavirus, and also pouchitis. More research is needed to clarify the role of probiotics for preventing antibiotic-associated diarrhoea, *Clostridium difficile* infection, travellers' diarrhoea, Irritable Bowel syndrome, Ulcerative colitis, Crohn's disease, and vulvovaginal candidiasis [25]. The condition and function of gastrointestinal (GI) tract are necessary for our wellbeing. Probiotics are defined as the microbial food supplements, which beneficially affect the host by improving and maintaining its intestinal microbial equilibrium. Globally probiotics are extensively used because it can be contemplated as functional food. They are used therapeutically to improve lactose tolerance and also to prevent diarrhoea (especially viral diarrhoea in infants, antibiotic-associated diarrhoea, *Clostridium difficile*-associated diarrhoea and Traveller's diarrhoea). Clinical studies suggest that probiotics might be useful for stimulation of the immune system, prevention of atopic eczema, control of gut inflammatory diseases (IBS, IBD) and cancer prevention (anticarcinogenic). Probiotic microbial species act by altering the constitution of the gut microbiota. The use of non-digestible oligosaccharides (prebiotics) can fortify intestinal microflora and also stimulate their growth. Many reviews have provided information regarding probiotic microbial strains, prebiotics and commercially products (functional foods), which can be thus useful in improving our health [26].

Thus, at the end of this study it can be summarized that packed curd, loose curd and medicinal probiotics all can contain pathogenic bacteria and yeasts, and the bacteria can well be acid fast bacteria and anaerobes also. Hence these things are of tremendous public health importance, and also highly relevant to food science also. The pH of the curd can have a bearing with the

pattern of bacteria present. As far as we know, these kinds of studies have not been carried out earlier in our country. Further public health or food science research should delve deeper in these aspects.

Conclusion

Hence this study may help us in ascertaining the burden of normal and pathogenic bacteria in probiotics and this will give a new direction and perspective to nutrition and public health research. A new formulation of tomato juice agar was also tested, and established, which can create a new dimension of public health research and food microbiology. These aspects are important but somewhat neglected. As far as we know, this type of study has not been carried out earlier. So more such studies are needed in this interrelated field of nutrition, infection and public health. Consuming curd and medicinal probiotics may occasionally be dangerous since they may contain pathogens. Their contents should be meticulously cross-checked before licensing them to be sold openly in market.

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