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Yeast probiotics fermented food products: Gut microbiome and Women health

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Abstract

Probiotics are defined as viable microorganisms that exhibit a beneficial effect on the hosts health when they are ingested. Probiotics, beneficial bacteria, can positively influence various aspects of women's health by promoting a balanced vaginal microbiome, supporting digestive health, and potentially impacting mood and immune function. Specifically, they can help to prevent and treat vaginal infections like bacterial vaginosis and yeast infections, and may play a role in supporting fertility and pregnancy outcomes. During pregnancy, probiotics may be helpful in reducing the risk of gestational diabetes, maternal group B streptococcal colonization, obstetric anemia, and postpartum mastitis. Therefore, probiotics represent a promising treatment option for a range of gynecological conditions. Overall, *Lactobacilli* and *Bifidobacteria* are indicators of a healthy vaginal microbiome, but there are limited data to support whether using probiotics to replenish either of these species can improve vaginal health and alleviate infections. ***Ayurveda*** recognizes the importance of gut health and emphasizes the use of probiotic-rich foods to promote digestion, immunity, and overall well-being. Several clinical investigations have declared S. cerevisiae var. boulardii a biotherapeutic agent due to its antibacterial, antiviral, anti-carcinogenic, antioxidant, anti-inflammatory and immune-modulatory properties. Yeast probiotic feremented food are very popular in the Indian market induce health-promoting effects in the host body helps in the gut heath. The gut microbiome is a viable therapeutic target for managing allergic diseases, as they modulate the immunological and inflammatory response that consequently affects the development of sensitization and allergy. The advantages of probiotics are related to the modulation of gut microbiota, mitigation of nutritional intolerances (lactose intolerance), increase in bioavailability of macro and micronutrients, and alleviation of allergic incidences in susceptible individuals. The gut plays a pivotal role in the digestion and absorption of nutrients and maintains mucosal barrier integrity.

Keywords: Bifidobacterium; Bacterial vaginosis; Microbiota; Probiotic; Saccharomyces cerevisiae var. boulardii; Vaginal infections; Vaginal dysbiosis; Yeast

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1. Introduction

Probiotics are defined as live microorganisms which when administered in adequate quantity confer health benefits to the host [1-15]. Probiotic bacteria, generally incorporated into fermented dairy products, mainly belong to the genera *Lactobacillu*s and *Bifidobacterium* [1-12, 74-80]. Two important criteria are used for selection of probiotic microorganisms must be able to survive in the gastrointestinal environment and to present at least one beneficial function (colonization resistance, immunomodulation or nutritional contribution) [1-35-75]. Probiotics are food or pharmaceutical preparations containing live non-pathogenic microorganisms which improve one of the three main beneficial functions (colonization resistance, immunomodulation or nutritional contribution) of the normal gastrointestinal microbiota, when ingested by human or animal hosts [1-45]. For this reason, most of the probiotics studied or commercialized today has been selected from the digestive ecosystem[1-45]. Various mechanisms of action have been proposed to explain *S. boulardii* protection. The use of yeasts as dietary supplements is still limited [1-45]. Some yeast strains, *viz*., *Saccharomyces cerevisiae* and *Saccharomyces boulardii* have been reported as probiotics in humans for many years as they are exerting some influence on the intestinal flora [1-45]. It was demonstrated that yeast modulates the immune system, degrades *C. difficile* toxins A and B and their respective receptors on colonic mucosa, inhibits cholera toxin action, modulates the transduction pathway induced by enteropathogenic *Escherichia coli,* stimulates digestive enzymatic activities and fixes some enterobacteriaceae on its surface [1-55]. Adhesion to the intestinal mucosa is one of the main selection criteria for probiotics [1-50]. Autoaggregation is clumping of microbes which belong to the same strain, while coaggregation is the result of cell-to-cell recognition between two different microbial strains [1-45]. Autoaggregation of probiotic strains has been correlated with adhesion to intestinal epithelial cells, known to be a prerequisite for colonization and enhanced persistence in the gastrointestinal system [1-45]. Coaggregation abilities may form a barrier that prevents colonization by pathogenic microorganisms [1-50]. Several studies have been conducted using human epithelial cell lines *viz.* HT-29, HT-29MTX, and Caco-2 to screen the adhesion capacities of probiotic strains [1-58]. The absence of hemolytic activity is considered as a safety prerequisite for the selection of a probiotic strain [1-58]. Hemolytic activity, urease activity, and cytotoxicity tests were evaluated for the characterization of the strains as potential probiotics [1-58].

Saccharomyces cerevisiae var. *boulardii* is best known for its treatment efficacy against different gastrointestinal diseases[57, 58-65]. This probiotic yeast can significantly protect the normal microbiota of the human gut and inhibit the pathogenicity of different diarrheal infections[57]. Several clinical investigations have declared S. cerevisiae var. boulardii a biotherapeutic agent due to its antibacterial, antiviral, anti-carcinogenic, antioxidant, anti-inflammatory and immune-modulatory properties[57]. Oral or intramuscular administration of *S. cerevisiae* var. boulardii can remarkably induce health-promoting effects in the host body[57, 58-65]. Different intrinsic and extrinsic factors are responsible for its efficacy against acute and chronic gut-associated diseases [57, 58-65].

Yeasts that have been classified as generally regarded as safe (GRAS) have been shown to have several health implications on the human host. These influences may include but are not limited to being effective on gut microbiota dysbiosis and possess anti-inflammatory, anti-proliferative, anti-cancer and anti-allergenic properties [5,17].Yeasts have been studied and have proven to be effective starter cultures, and significant interest has been noted in their use in various biotechnological applications [57, 58-65]. Yeasts make up <0.1% of the human microbiome. Most yeast isolates that have been isolated from the human microbiome include *Candida albicans*, *Torulopsis glabratra, Candid tropicalis, Malassezia* spp., and *Saccharomyces* spp [57, 58-65]. Other probiotic yeast candidates include *Cryptococcus spp., Candida famata, C. tropicalis, Debaryomyces hansenii, Issatchenkia orientalis, Kluyveromyces lactis , Kluyveromyces marxianus, Metschnikowia gruessii, Pichia jadinii, Pichia kluyveri, Pichia kudriavzevii , Pichia pastoris, Pichia guilliermondii,* and *Wickeramomyces anomalus* [16]. The ability to resist low pHs, the presence of digestive enzymes, bile salts, and organic acids make these organisms ideal candidates to serve as probiotics [57, 58-65].

Saccharomyces boulardii, Saccharomyces cerevisiae, and *Candida* spp. are the most common yeasts used as probiotics, and are used most for the treatment of Clostridium difficile diar- rhea [1-65]. *S. boulardii* was first isolated from litchis in Indochina and is not autochthonous in the microbiome [2-50]. However, this non-pathogenic yeast, amongst other species such as *Saccharomyces cerevisiae, Pichia kudriavzevii, Candida famata, Kluyveromyces lactis, Debaryomyces hansenii*, and *Issatchenkia orientalis*, have been found to confer the following probiotic effects to the host, advocating their use for health benefits [65].

2. Human Gastrointestinal (GI) tract

The gastrointestinal (GI) tract, also known as the digestive tract or alimentary canal, is the series of hollow organs and passage ways that extends from the mouth to the anus (Figure-1)[66-69-152]. The GI tract plays a vital role in breaking down food, absorbing nutrients, and eliminating waste [66-69]. The GI tract is a series of hollow organs joined in a long, twisting tube from the mouth to the anus [66-69]. The digestive tract is made up of organs that food and liquids travel through when they are swallowed, digested, absorbed and leave body as feces[66-69]. The GI tract is a series of hollow organs joined in a long, twisting tube from the mouth to the anus. These organs include the mouth, pharynx (throat), esophagus, stomach, small intestine, large intestine, rectum and annus (Figure-1) [66-69]. The liver, pancreas, and gallbladder are the solid organs of the digestive system [66-69]. The digestive system includes the GI tract and the accessory organs of the liver, gall bladder and pancreas [66-69-152].

The gastrointestinal (GI) tract, also called the digestive tract or the alimentary canal, is the system of organs within multicellular animals that takes in food, digests it to extract energy and nutrients, and expels the remaining waste[66-69]. The major functions of the GI tract are digestion facilitated by motility, secretion and absorption [66-69]. The various patterns of GI tract function are generated by the integrated behaviour of multiple tissues and cell types[66-69]. Medical imaging methods such as ultrasonography, magnetic resonance imaging (MRI), and endoscopic ultrasound (EUS) are well known stand-alone clinical methods that can disclose structural and functional abnormalities of the GI tract[66-69-152]. Our digestive system is made up of a series of organs that allows our bodies to absorb nutrients and water from the food we eat[66-69]. As food travels through the digestive system it is broken down, sorted, and reprocessed before being circulated around the body to nourish and replace cells and supply energy to our muscles[66-69]. Digestion starts in the mouth where chewing and saliva breaks down food so it is more easily processed by our body[66-69-152].

The GI tract is divided into the upper GI tract, which runs from the mouth to the stomach, and the lower GI tract, which includes the small and large intestines[66-69]. Together, the GI tract and accessory organs use mechanical digestion and chemical digestion to break down food [66-69]. When we eat, our body turns the food into energy and extracts vitamins, minerals and other nutrients to help it work properly[66-69-152]. This process is called digestion. The parts of the body that are involved in digestion are called the digestive system. The central part of the digestive system is a winding muscular tube called the GI (gastrointestinal) tract[66-69]. Other parts of the digestive system, called accessory organs, help the GI tract to digest food. They include the: liver, gall bladder, pancreas[66-69]. The GI tract, or digestive tract, has two main parts: the upper digestive tract, the intestines. The upper digestive tract includes the mouth, the esophagus and the stomach [66-69]. The mouth is where digestion begins [66-69]. Even before we eat, the sights and smells of food trigger salivary glands in our cheeks and jaw to release saliva [66-69]. Saliva has two roles when we eat[66-69]. It contains digestive juices called enzymes to break down the starch in food. It helps to form the food into a compact "glob" called a bolus in our mouth[66-69]. This makes the food easier to swallow[66-69]. When food leaves the mouth, it passes through the pharynx into the esophagus. The esophagus is the muscular tube that gradually pushes food down to the stomach. It does this through waves of contractions known as peristalsis[66-69]. The wall of the esophagus releases a thick, sticky liquid called mucus[66-69-152]. This mucus helps the body to absorb the food we eat. It also lubricates the esophagus so food moves easily to the stomach. The stomach is a bean-shaped, hollow muscular organ that contains digestive acids[66-69]. These acids help to break down food some more and turn it into liquid. The walls of the stomach are thick and elastic. The size of the stomach depends on a person's size and body type and how much and how recently they have eaten[66-69]. The upper part of the stomach mainly stores food and relaxes to allow food to enter from the esophagus [66-69]. In the lower part of the stomach, food is broken down through mechanical and chemical digestion. Mechanical digestion means that the stomach mixes, churns and pummels food using its muscles. This turns the food into a thick liquid paste called chyme[66-69]. Chemical digestion uses gastric juice to break down the protein in food [66-69]. This gastric juice is a mix of chemicals and water and is very acidic[66-69]. These processes account for part of digestion. The rest of digestion happens in the intestines. Small intestine: Food first passes into the small intestine[66-69]. This long, hollow tube breaks down food through mechanical and chemical digestion and allows the food to pass into our blood [66-69]. The small intestine gets its name only because it is narrow[66-69]. In fact, in an average adult, it measures about 22 feet (seven metres) [66-69]. The small intestine has three parts[66-69]. The duodenum is where most of the remaining chemical digestion takes place[66-69]. Chemicals and digestive juices from the liver, gall bladder and pancreas help in this process. The jejunum is where carbohydrates and proteins pass into the blood[66-69-152]. The ileum is where vitamin B12 and bile salts pass into the blood. By this time, food has been broken down into its smallest, most basic units and is ready to be absorbed[66-69]. The small intestine has a large surface area for this function due to its special folds and tiny, finger-like projections, called villi [66-69]. Once food passes through the walls of the small intestine, it separates [66-69]. Carbohydrates, proteins and some fats go to the liver to be processed [66-69]. The remaining fats go into the blood[66-69]. Large intestine: The large intestine is sometimes called the colon. In adults, it is about five feet (1.5 metres) long[66-

69]. Its role in digestion is to absorb the water from any leftover undigested food[66-69]. It is also home to gut flora, which help us to digest food and protect us from infections. Three bands of muscle on the surface of the large intestine move waste products along by waves of contractions called mass movements[66-69]. When there is enough waste material in the colon, further contractions push the feces (poo) into the rectum before it is released through the anus[66-69-152].

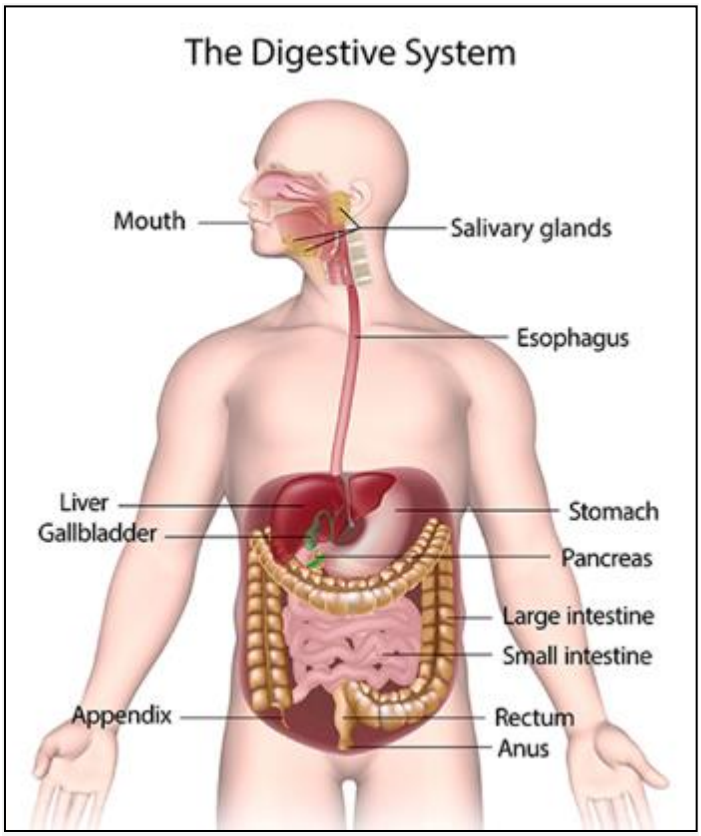

Figure 1 The digestive tract

3. Gut Microbiome

The gut microbiome refers to the vast community of microorganisms, including bacteria, viruses, fungi, and other microbes, that live in the digestive tract (Figure-1) [70-72-152]. This complex ecosystem plays a vital role in human health, influencing digestion, immune function, and even brain health[70-72]. Given the close symbiotic relationship existing between the gut microbiota and the host, it is not surprising to observe a divergence from the normal microbiota composition (generally referred to as dysbiosis) in a plethora of disease states ranging from chronic GI diseases to neuro developmental disorders [70-72-152]. Human body is a natural host for a variety of microbiomes, which are communities of microorganisms that inhabit different regions of the body. It has been estimated to have trillions of microorganisms, including bacteria, viruses, fungi, and other microbes, living in and on the human body. These microbiomes are found in various parts of bodies, including the gut, skin, oral cavity, respiratory tract, urogenital tract, and other sites. The composition and diversity of these microbiomes are known to be important for human health, and a disruption in their balance has been associated with various diseases and conditions [70-76]. Microbiota describes the living microorganisms found in a defined environment, such as oral and gut microbiota [70-72]. Microbiome refers to the collection of genomes from all the microorganisms in the environment, which includes not only the community of the microorganisms, but also the microbial structural elements, metabolites, and the environmental conditions [70-72]. In this regard, microbiome encompasses a broader spectrum than that of microbiota. The gut microbiota is now considered as one of the key elements contributing to the regulation of host health [70-72]. Virtually all our body sites are colonised by microbes suggesting different types of crosstalk with our organs [70-72]. Because of the development of molecular tools and techniques (ie, metagenomic, metabolomic, lipidomic, metatranscriptomic), the complex interactions occurring between the host and the different microorganisms are progressively being deciphered [70-72-152]. Now a days, gut microbiota deviations are linked with many diseases including obesity, type 2 diabetes, hepatic steatosis, intestinal bowel diseases (IBDs) and several types of cancer [70-72]. Thus, suggesting that various pathways

involved in immunity, energy, lipid and glucose metabolism are affected. In healthy subjects, the oral and saliva microbiomes contain millions of microbes that are swallowed daily with our food, but their persistence in the gut is impeded by many factors, including the acidity of the stomach, the production of bile acids (BAs), digestive enzymes and antimicrobial proteins in the duodenum and beyond [70-72-76]. Probiotics function through the restoration of equilibrium within the gut microbiota, the establishment of beneficial bacterial colonies, and interactions with immune cells within the gut-associated lymphoid tissue. These actions collectively enhance processes such as digestion, skin health, immune system function, and can also influence mental health via modulation of the gut-brain axis[70-76].

Yeast probiotics, particularly *Saccharomyces boulardii*, play a significant role in the gut microbiome by promoting a healthy balance of microorganisms and potentially alleviating gastrointestinal issues [1-70-75]. These yeasts act as temporary shields in the gut, preventing harmful bacteria from overgrowing and aiding in the reestablishment of a balanced microbiome. *S. boulardii* is well-studied and has shown efficacy in treating and preventing various gastrointestinal disorders [1-70-75]. *Saccharomyces cerevisiae* var. *boulardii* is best known for its treatment efficacy against different gastrointestinal diseases[1-70-73]. This probiotic yeast can significantly protect the normal microbiota of the human gut and inhibit the pathogenicity of different diarrheal infections. Several clinical investigations have declared *S. cerevisiae* var. *boulardii* a biotherapeutic agent due to its antibacterial, antiviral, anti-carcinogenic, antioxidant, anti-inflammatory and immune-modulatory properties [1-70-75]. Oral or intramuscular administration of *S. cerevisiae var. boulardii* can remarkably induce health-promoting effects in the host body. Different intrinsic and extrinsic factors are responsible for its efficacy against acute and chronic gut-associated diseases[1-70-75].

4. Probiotic Fermented Food

The use of probiotics has been gaining popularity in terms of inclusion into human diets over recent years [1-39, 65]. Based on properties exerted by these organisms, several benefits have been elucidated and conferred to the host. Bacteria have been more commonly used in probiotic preparations compared to yeast candidates; however, yeast exhibit several beneficial properties, such as the prevention and treatment of diarrhea, the production of antimicrobial agents, the prevention of pathogen adherence to intestinal sites, the maintenance of microbial balance, the modulation of the immune system, antibiotic resistance, amongst others [65]. *Saccharomyces boulardii* is by far the most studied strain; however, the potential for the use of other yeast candidates, such as *Kluyveromyces lactis* and *Debaryomyces hansenii*, amongst others, have also been evaluated [65].

In one of the study, reported by Wang et al. (2024) [1], the probiotic potential of yeast strains isolated from commercial kombucha produced in New Zealand were evaluated *in vitro* [1]. Four yeast strains of *S. pombe* LBY5, *B. anomalus* DOY8 *P. kudraivzevii* GBY1, *S. cerevisiae* GBY2 exhibited excellent tolerance to simulated GI conditions (low pH, human temperatures, and presence of bile salts), cell surface hydrophobicity, auto-aggregation, co-aggregation with pathogenic bacteria, and antioxidant activity[1]. Results of study conducted by Wang et al. (2024) [1] showed that all the 15 strains tested in this study can be regarded as safe for consumption based on the absence of hemolysis, proteolytic, phospholipase and gelatinase activities[1]. Strains of *P. kudraivzevii* GBY1 and *S. cerevisiae* GBY2 showed resistance to all tested antibiotics[1]. Thus, these two yeast strains, (*P. kudraivzevii* GBY1 and *S. cerevisiae* GBY2) may be promising novel probiotic yeast for the food industry due to their excellent antioxidant activity and cell surface characteristics[1]. However, *in vivo* testing, to analyse the health-promoting benefits of these probiotic strains as well as their stability during storage are recommended [1].

In one of the study reported by Malabadi (1994) [36], Malabadi and Raghvendra (1994) [37], Malabadi and Raghavendra (1995) [35], sixty yeast were isolated from Dharwad, Karnataka State, India of different substrates such as flowers, fruits, soil, biodegrading materials and sugary substances [35, 36, 37]. Ten yeast isolates were identified as *Candida utilis, Torulopsis versatilis, Pichia pinus, Saccharomyces cervisiae, Candida rhagii, Cryptococcus luteolus, Saccharomyces fermentatis, and Kluyveromyceas* species [35, 36, 37]. Samples of natural substrates such as nectars of flowers, fruits, dry raisins, insects like hony bees, decying organic materials, molasses, jaggery, honey and water samples were collected [35, 36, 37]. Samples were transferred to 1ml sterile vials. By adopting the techniques of pour plate method and serial dilution methods, the microorganisms were cultured on petridishes containing Wickerhams medium (Yeast Malt Agar (YM Agar medium) [35, 36, 37, 59, 60, 61, 62]. The YM agar medium [61, 62] was consisting of 3g/ lit of yeast extract, 3g/lit of malt extract, 5g/lit of peptone, 10g/lit of glucose, and 20g/lit of agar. The pH of the medium was 4.5 adjusted using diluted HCL [35, 36, 37]. The media was sterilized at 121 ^{o} C for 15 min. The inoculated Petri dishes were incubated at 28 ^{o} C for 72 hours [35, 36, 37]. The growth of microorganisms was observed microscopically and on the basis of observation, yeast colonies were marked and separated [35, 36, 37]. The yeast isolates were identified on the basis of methods of Lodder, 1970 **[59]** and Barnett et al., 1983 [60]. Yeast isolates were identified on the basis of morphological, cultural, sexual, and physiological characteristics including Diazonium blue salt (DBB test), urease test, cycloheximide (Actidione reaction test), assessing vitamin requirements and fermentation ability of

different carbon sources [35, 36, 37, 59, 60]. Wickham's medium, also known as Yeast Malt Agar (YM Agar), is a non-selective growth medium primarily used for cultivating yeasts, molds, and other aciduric microorganisms. It's formulated with yeast extract, malt extract, peptone, and agar. YM Agar can be further modified to enhance selectivity by acidification or adding antibiotics [35, 36, 37, 59, 60-63].

In New Zealand, Kombucha is a popular, slightly sweet, sparkling drink fermented for 7–10 days at ambient temperature in a base of sugared tea infusion, typically made with black or green tea[1]. Although many commercial kombucha products are labelled as containing "live cultures", their probiotic properties have not been documented [1]. Probiotics are defined as live microorganisms with appropriate concentrations of well-defined strains which exhibit health-promoting benefits to the host [1-35]. To confer health benefits, probiotic microorganisms must be able to survive in the dynamic gastrointestinal environment, which includes low pH and pepsin in the stomach and bile salts and pancreatic enzymes in the intestine[1-40]. Additionally, they must be capable of propagating at human body temperatures [1-50]. Therefore, several selection criteria are essential for microorganisms to be considered as probiotics, including being safe for consumption and producing non-toxic activities to the human body [1-55]. A safety assessment of any potential probiotic strain must be conducted before the culture can be considered for use in food or pharmaceutical products [1-45]. The ability of probiotics to adhere to epithelial cells is a desirable characteristic of beneficial microorganisms allowing them to manifest their beneficial effects by persisting longer in the host GI tract [1-50]. Antioxidant and antimicrobial activities have also been used to identify potential probiotic strains [1-50]. Most probiotics are lactic acid bacteria (LAB) with the majority belonging to the genera *Lactobacillus, Enterococcus and Bifidobacterium* [1-46]. However, to date only a few yeast strains have been identified as probiotics [1-50]. *Sacchromyces cerevisiae* and *S. boulardii* are the only commercial probiotic yeast species currently available for human consumption [1-55]. Yeast is commonly found in food and beverages and are utilised as starter cultures for fermentation of several food and beverages such as wine, kombucha, lambic beer, kefir, sake, bread-making, idli, dosa, buttermilk, youghurt, and table olives [1-55]. Their wide applications in the food industry indicated that the most food-related fungi (yeast) are generally regarded as safe [1-40]. Recently, consideration of yeast as potential probiotic microorganisms has increased due to their advantages over bacteria [1-55], for example, the larger size of yeast cells (approximately 10 times larger) compared to bacteria allowed them to exhibit stearic hindrance to bacteria [1-55]. Yeast such as the genera *Pichia, Schizosaccharomyces, Kluyveromyces, Yarrow,* and *Torulaspora* have shown several beneficial characteristics for health, including excellent resistance to antibiotics which helps to restore gut microbiota after antibiotic administration[1-55]. Yeast is also characterised by having a high mineral and vitamin B content, the presence of several immune response components in their cell wall such as mannose and glucan, and ability to grow under conditions similar to the harsh GI tract environment [1-50]. Despite a large number of probiotics being used in commercial products, the rapid growth of the probiotic market indicates that demand exists for more strains possessing specific functional properties such as antioxidant and anti-inflammatory activities [1-55]. Therefore, it is necessary to determine the characteristics of other potential probiotic yeast species or strains from food or beverage sources [1-50]. Kombucha is generally regarded as a probiotic drink due its inherent live microorganisms [1].

In another study by RAGAVAN and DAS (2017) [11], five yeast strains were identified at the molecular level and found to possess the desirable *in vitro* probiotic properties based on their autoaggregation, coaggregation, and nonhemolytic capacities. Being non producer of urease, all the yeast strains were confirmed as safe bioresources [11]. *In vitro* studies on cytotoxicity with SRB demonstrated that no toxic substance was produced by the probiotic yeast strains [11]. Therefore, the identified yeast strains *viz*. *Y. lipolytica* (LM), *K. lactis* (MR), *L. starkeyi* (GOI), *S. fibuligera* (GII2), and *B. custersianus* (WI) can be used as potential probioticsin functional foods and health-related products [11]. In this study by RAGAVAN and DAS (2017) [11], the yeast strains, *viz*., LM, MR, GOI, GII2, and WI was identified at the molecular level and named as *Yarrowia lipolytica* VIT-MN01, *Kluyveromyces lactis* VIT-MN02, *Lipomyces starkeyi* VIT-MN03, *Saccharomycopsis fibuligera* VIT-MN04, *Brettanomyces custersianus* VIT-MN05, respectively [11]. Maximum autoaggregation (92%) and coaggregation (97 %) were noted in case of *L. starkeyi* VIT-MN03[11]. All yeast strains showed non-hemolytic activity. *In vitro* toxicity assay was performed and all the yeast strains showed nontoxic nature[11]. RAGAVAN and DAS (2017) [11] reported that five yeast strains have been studied for their probiotic characteristics and identified at molecular level [11]. Out of five yeast strains, three strains showed maximum adhesion ability, which is a prerequisite for colonization and protection of gastrointestinal tract [11]. All the yeast strains are validated as a safe bioresources because of their non-hemolytic activities and nonproduction of urease [11]. This study by RAGAVAN and DAS (2017) [11] concluded that the identified yeast strains can serve as promising probiotics in various fields of food industry [11].

According to the latest definition of the World Health Organization, probiotics are active microbes that stimulate the growth of other probiotic bacteria in the gut and possess beneficial health effects to the host [57]. These microorganisms are able to produce anticarcinogenic, antioxidant and anti-mutagenic agents and induce protection against different bacterial diseases including diarrhea and respiratory tract infections[57]. *Saccharomyces cerevisiae* var. *boulardii* is the

most significant probiotic yeast species. S. cerevisiae var. boulardii is a eukaryotic organism that has been used in scientific investigations since the time of its discovery [57-65]. Fermented foods are a source of diverse microflora and the fact that very few types of yeast have been commercially used as probiotic, it is worthwhile to explore the diversity of yeasts in traditional Indian fermented foods and study their probiotic attributes [4]. *Saccharomyces boulardii*, a yeast strain isolated about a hundred years ago, is the most well-characterized probiotic yeast [56].

In one of the study reported by Syal and Vohra (2012) [4], twenty yeast strains were isolated from traditional Indian fermented foods (idli and jalebi batter) and were screened for various probiotic properties. According to the study reported by Syal and Vohra (2012) [4], seven of these isolates could survive in conditions similar to the gut with a survival rate as high as 100% at pH 2.0-2.5 and bile salt concentration of 1% [4]. They were able to grow at 37ºC and were resistant to commonly used antibiotics[4]. Syal and Vohra (2012) [4] also reported that auto-aggregation ability and cell surface hydrophobicity was observed to be high for all of the isolates [4]. Antimicrobial action was exhibited by these isolates against enteric pathogens (i.e., *E. coli*, *Salmonella* sp., *Staphylococcus aureus*, *Vibrio cholerae* and *Pseudomonas* sp.) [4]. Syal and Vohra (2012) [4], also reported that they were observed to produce phytase, β-galactosidase, L-asparaginase, protease and lipase, which could be useful in degrading anti-nutrients and improving digestion [4]. Most of them were vitamin B12 (except J15) and exopolysaccharide producers [4]. All of them had the ability to assimilate cholesterol in the range 57-88.5%[4]. None of the strains produced DNase and gelatinase, thus ascertaining their safe use[4]. These isolates were identified as *Saccharomyces cerevisiae*, *Candida tropicalis*, *Aureobasidium* sp. and *Pichia manschuria* [4]. They also produce L-asparaginase which has anticancer property [4]. These yeast isolates could aid in digestion because of their ability to produce lipase and protease[4] . Enhance vitamin B12 pool due to its production by these isolates. They can be used as immune-stimulants[4]. Their ability to assimilate cholesterol could be of immense value to patients suffering from hypercholesterolemia [4]. Beneficial attributes of *Saccharomyces cerevisiae*, *Candida* sp., *Pichia* sp., and *Aureobasidium* sp. indicated that these yeasts are promising probiotic agents in future and can be widely used as food and feed supplements[4]

According to the study conducted by Azhar and Munaim (2019) [9], the majority of the bacterial and yeast strains that can be found in the kefir drink in Malaysia are *Lactobacillus* and *Saccharomyces* that are recognised as probiotic microorganisms [9]. In Malaysia, Kefir drink is a product from the fermentation process of milk using symbiotic mixture of bacteria and yeast consortium [9]. *Saccharomyces* and *lactobacillus* are the major genera found in the kefir drink [9]. Kefir drink is a product that undergoes a fermentation process using milk as a medium [9]. The taste is quite acidic and it has a creamy-like texture [9]. It is normally produced from a traditional kefir grains or kefir starter cultures by fermentation process[9]. Kefir comprises a microbial symbiotic mixture of bacteria and yeast that attached to a polysaccharide matrix [9]. Although the bacterial population is dominant in kefir, the presence of yeast plays an important role to develop the flavour as well as chemical composition of the kefir product [9]. Moreover, the yeast strains are important for the microbial balance by providing the essential nutrients for a probiotic bacteria population such as vitamins, amino acid and produce some compounds that contribute to the kefir drink taste[9]. Azhar and Munaim (2019) also reported the isolation and identification of potential probiotic yeast strains in the kefir drink samples from Malaysia[9] . The molecular identification was done by PCR using ITS1 and ITS4 amplified regions[9]. Nine different yeast strains were isolated, and the strains were successfully identified based on the sequence analysis[9]. *Saccharomyces* and *Kodamaea* were found to be the major population in the kefir drink samples[9]. Lastly, kefir milk is one of the excellent sources of probiotic yeast strains and could be used as a new yeast probiotic formulation or in food supplements[9]. Moreover, the amplification of ITS region can be used as a useful method to identified yeast strains[9]. Internal transcribed spacer (ITS) refers to the spacer DNA situated between the small-subunit ribosomal RNA (rRNA) and large-subunit rRNA genes in the chromosome[9]. It contains two noncoding spacer regions between 18s and 28s including 5.8s rRNA gene [9]. In yeast and fungal species, the universal primers ITS1 and ITS4 are commonly used to amplify the region[9]. The yeast strains found could be used as a new source of probiotic formulation such as in tablet or capsule form or can be incorporate in the supplement or functional food [9]. Following are few Indian probiotic fermented food in the market[74, 75, 154].

- **Curd or Yoghurt** is one of the most popular probiotic food in India. It is made by fermenting milk with lactic acid bacteria, which helps to maintain a healthy balance of gut flora. Consuming curd regularly can aid digestion, boost immunity, and prevent stomach-related issues common during the monsoon [4-12, 27, 35-39, 41-48, 52]. Enjoy it plain, or use it in dishes like Raita, Lassi, or Kadhi [154].
- **Buttermilk,** or chaas, is a refreshing probiotic drink made by diluting curd with water and adding spices like cumin and coriander. It is light on the stomach and helps in cooling the body. Buttermilk aids in digestion and can alleviate bloating and acidity, making it an excellent choice for maintaining gut health during the humid monsoon season [4-12, 27, 35-39, 41-48, 52].
- **Idli and dosa** are traditional South Indian fermented foods made from rice and urad dal batter. The fermentation process enhances the nutritional value and makes these foods rich in probiotics[154]. Including

idli or dosa in our diet can improve digestion and provide beneficial bacteria to the gut, promoting overall digestive health [4-12, 27, 35-39, 41-48, 52].

- Traditional Indian **pickles, or achaar**, are made by fermenting vegetables and fruits with spices and salt. The fermentation process produces beneficial bacteria that can aid in digestion and improved gut health[154]. Pickles made from ingredients like mango, lime, and green chili are not only flavourful but also rich in probiotics [4-12, 27, 35-39, 41-48, 52, 74, 75].
- **Kanji** is a traditional North Indian probiotic drink made from fermented black carrots or beets. It is typically consumed during the winter and early spring, but it can be a great addition to our monsoon diet as well [4-12, 27, 35-39, 41-48, 52]. Kanji is rich in beneficial bacteria and enzymes that promote a healthy digestive system. Its tangy taste is both refreshing and nutritious [154].
- **Gundruk** is a traditional fermented leafy green vegetable dish from the Himalayan region, particularly popular in Nepal and some parts of India. It is made by fermenting leafy greens like mustard, radish, or cauliflower leaves. Gundruk is a rich source of probiotics and can help to improve digestion and boost gut health, especially during the monsoon [4-12, 27, 35-39, 41-48, 52].
- **Sol Kadhi** is a traditional probiotic drink from the Konkan region of Maharashtra and Goa. It is made from kokum fruit and coconut milk, sometimes with the addition of fermented buttermilk[74, 75, 154]. Sol Kadhi is not only a cooling beverage but also aids in digestion and soothes the stomach, making it a perfect drink for the monsoon [74, 75, 154].
- **Fermented rice**, known as Panta Bhat in Bengali and Poita Bhat in Assamese, is a traditional dish where leftover cooked rice is soaked in water overnight to ferment. The fermented rice is consumed the next day with salt, onions, and green chilies. This dish is also rich in probiotics and helps to improve digestion and boost gut health. [4-12, 27, 35-39, 41-48, 52].
- **High-fiber fruits: Bananas**: This versatile fruit is rich in resistant starch, a type of fiber that feeds beneficial bacteria. They are particularly beneficial when unripe and widely consumed across the country[154].
  - **Apples**: Besides offering a crunchy delight, apples contain pectin, a soluble fiber that supports a healthy gut by encouraging the growth of good bacteria and keeping the harmful ones in check.
  - **Guava**: This tropical fruit is a fibre powerhouse, containing both soluble and insoluble fiber to keep our digestive system running smoothly[154].
- **Root vegetables: Carrots**: A classic Indian ingredient, carrots are a good source of prebiotic fiber that can support gut health[154].
  - **Beetroots**: These vibrant root vegetables contain beet fiber, which can benefit gut bacteria and improve digestion[154].
- **Legumes**: **Lentils**: These are integral to many Indian recipes and are high in fiber, particularly resistant starch, which is great for nurturing gut bacteria[154].
  - **Chickpeas**: Another staple in Indian meals, chickpeas contain soluble fibre that specifically feeds beneficial bacteria, fostering a healthy gut environment.
  - **Whole grains**: **Brown rice**: A healthier alternative to white rice, brown rice is rich in fibre, including resistant starch, which can benefit our gut[154].
  - **Jowar (sorghum)**: Rich in fiber, jowar supports a diverse microbiota and adds to the richness of Indian cuisine[4-12, 27, 35-39, 41-48, 52].
- Kanji and Kombucha are two probiotic beverages that have gained popularity in India for their health benefits and unique flavors[4-12, 27, 35-39, 41-48, 52, 154]. Kanji is a traditional drink from North India made by fermenting black carrots with water, mustard seeds, and other spices[4-12, 27, 35-39, 41-48, 52]. Kombucha is a fermented tea beverage made by fermenting sweetened tea with a culture of bacteria and yeast known as a SCOBY (symbiotic culture of bacteria and yeast). Preparing Kanji and Kombucha at home is a relatively simple process, with the black carrots grated and mixed with water, mustard seeds, and spices, and then left to ferment for a few days [4-12, 27, 35-39, 41-48, 52]. Kombucha requires brewing a batch of sweetened tea and allowing it to ferment for about a week. Incorporating Kanji and Kombucha into our diet can be a refreshing and beneficial choice[4-12, 27, 35-39, 41-48, 52]. These probiotic beverages can be consumed independently as a healthy alternative to sugary drinks or paired with meals for added flavor and digestive support[154].
- **Miso (Soybean Fermentation):** While miso is not Indian in origin, it has gained popularity and is used in some Indian recipes. Miso is a traditional Japanese seasoning made by fermenting soybeans with salt and a specific mould called koji. It can be added to soups, stews, and dressings to introduce probiotics into our diet.
- **Dhokla** is a popular Gujarati snack made from fermented chickpea flour. The fermentation process adds probiotic bacteria, improving gut health. It enhances the taste, texture, and nutritional value of dhokla [4-12, 27, 35-39, 41-48, 52]. During the fermentation process, the bacteria consume the carbohydrates in the batter, producing lactic acid as a by-product[4-12, 27, 35-39, 41-48, 52, 154]. This lactic acid not only adds a tangy

taste but also helps in breaking down complex nutrients, making them more easily digestible [4-12, 27, 35-39, 41-48, 52]. Additionally, the lactic acid bacteria produce certain enzymes and vitamins that further enhance the nutritional value of dhokla[154].

- **15) Kefir: l**ike Miso, Kefir is not originally Indian but has gained popularity and is consumed in some parts of India. It is a fermented milk drink made using kefir grains, which contain a combination of bacteria and yeast. Kefir is known for its probiotic properties and is often consumed as a health drink. These Indian probiotics not only tantalize the taste buds but also contribute to gut health, immune function, improved digestion, and overall well-being [4-12, 27, 35-39, 41-48, 52]. As awareness grows regarding the importance of a balanced gut microbiome, understanding the difference between probiotics and prebiotics becomes crucial. By incorporating Indian probiotic foods into our diets, we can embrace a flavorful and nourishing way to support our health [4-12, 27, 35-39, 41-48, 52, 154].
- All these probiotic foods maintain the balance of gut bacteria, strengthen immunity, and ensure overall health, making them simple and effective inclusions in our daily diet for improved gut health. Some of the top Indian probiotic foods and drinks which can have daily for a healthy gut: 1. Curd (Dahi) 2. Buttermilk (Chaas) 3. Kanji Lassi 5. Idli / Dosa 6. Dhokla 7. Handvo 8. Appam 9. Khusha 10. Khambral (Kombucha) 11. Pickles (Achaar) 12. Sauerkraut (Homemade) 13. Pakhala / Panta Bhat 14. Fermented Soy (Tempeh/Natto) 15. Khaman 16. Fermented Porridge 17. Kimchi (Indian-style) 18. Fermented Peanut Chutney 19. Ragi Malt (Fermented) 20. Kefir (Homemade) [1-154].

5. Yeasts influencing Plants Growth

Jeberlin Prabina et al., (2019) [33] reported that the benefits enjoyed by the plants up on inoculation with these beneficial microbes include improved availability of nutrients, reduced infection by pathogens and enhanced resistance to abiotic stress such as drought, temperature and salinity [33] . The common effects that could be realised in plants due to the beneficial microbes are improvement in germination, seedling vigor, biomass production, root hair development, photosynthetic efficiency, improved plant biochemical composition flowering, and yield[33]. Among the growth promoting microbes, beneficial bacteria commonly referred as Plant Growth Promoting Bacteria (PGPR) [33] are widely exploited and has got much attention. A diverse range of yeasts exhibit plant growth promoting characteristics, including control of pathogens [33], plant growth hormone production ; phosphorus solubilisation, nitrogen and sulphur oxidation and siderophore production [33]. The plant based yeasts *Issatchenkiaterricola*GRY4 and *Pichia kudriavzevii* POY 5 are multi-functional that they could solubilize phosphorus, zinc nutrients solubilization; produce auxin, siderophore, ACC deaminase and hydrogen cyanide that either directly or indirectly affect the plants[33]. Significant influence on vigor index of black gram on seed imbibition with the yeast isolates strongly substantiates the growth promoting nature of the yeast isolates [33].

6. Women Vaginal health influenced by Probiotics

Pagar et al., (2024) [149] are of the opinion that **vaginal health** is essential to a woman's overall well-being, as abnormalities in vaginal health can lead to a variety of gynaecological disorders, such as urinary tract infections, yeast infections, and bacterial vaginosis [74- 152]. The vaginal microbiome is essential for the prevention of these infections. Disruptions in this microbial ecosystem can significantly impact vaginal health[74- 152]. The concept of utilizing probiotics and prebiotics to stimulate the growth of protective vaginal microbiota has gathered substantial interest in recent years[74- 152]. Probiotics are live micro-organisms that strengthen and restore vaginal microbial balance by lowering pH levels, production of bacteriocins, biofilm disruption, modulation of immune response, and production of hydrogen peroxide (H_2O_2), consequently combating the development of pathogens. Prebiotics are oligosaccharides that encourage the development of probiotics such as lactobacilli species[74- 152]. Probiotics and prebiotics also have some broader implications for vaginal health, including their role in minimizing the incidence of premature birth, optimizing fertility, managing menopausal symptoms, and preventing vaginal infections [74- 152]. Synbiotics are a combination of probiotics and prebiotics that deliver additional benefits by encouraging the development and activity of beneficial microbes[74- 152]. Furthermore, postbiotics are bioactive compounds derived from probiotic bacteria during fermentation that have immunomodulatory actions and provide an additional layer of protection against vaginal infections. The present study highlights the most prevalent vaginal infections and limitations of existing therapies that influence the vaginal microbiota [74- 152]. The profound consequences of probiotics and prebiotics in women's health, including their role in minimizing the prevalence of vaginal infections and promoting overall vaginal health, as well as advanced therapeutic strategies such as synbiotics and postbiotics, are also discussed[74- 152]. The literature offers significant insights into the mechanism, efficacy, and safety of probiotics and prebiotics to healthcare providers and researchers[74- 153].

The first report to demonstrate that probiotics can be applied directly to the vagina was published in 1992 by Reid et al. [74, 76, 77-112-152]. Probiotics, beneficial bacteria, can positively influence various aspects of women's health by promoting a balanced vaginal microbiome, supporting digestive health, and potentially impacting mood and immune function [70-76]. Specifically, they can help prevent and treat vaginal infections like bacterial vaginosis and yeast infections, and may play a role in supporting fertility and pregnancy outcomes[70-76-152]. Probiotics, live microorganisms that confer health benefits to the host when administered in adequate amounts, have gained considerable attention for their potential role in maintaining women's health. Wu et al., (2024) [76] are of the opinion that probiotics, particularly *Lactobacillus* species, contribute to vaginal health by promoting a balanced vaginal microbiome to prevent infections and maintain an acidic environment [74, 76, 77-112-152]. In gynecologic conditions, probiotics showed potential in preventing and managing bacterial vaginosis, vulvovaginal candidiasis, and sexually transmitted infections[70-76-152]. Probiotic supplementation has also been associated with improvements in metabolic parameters and menstrual irregularities in polycystic ovary syndrome patients. During pregnancy, probiotics may be helpful in reducing the risk of gestational diabetes, maternal group B streptococcal colonization, obstetric anemia, and postpartum mastitis[70-76-112-152]. In recent years, the potential role of probiotics in the prevention and management of gynecologic cancer has gained attention. Further research is needed to better understand the specific mechanisms and determine the optimal Lactobacillus strains and dosages regimens for gynecologic cancer prevention and therapy [70-76-112-152]. Therefore, probiotics offer a non-invasive and cost-effective approach to support women's health and prevent obstetric and gynecologic complications[76-112-152]. These microorganisms are naturally found in certain foods, such as yogurt, idli, dosa, kefir, sauerkraut, kimchi, and kombucha, or in dietary supplements[70-76-153].

The vaginal microbiome is largely composed of bacteria, and the most common bacterial species found in the vagina are from the genus Lactobacillus. In healthy women of reproductive age [74, 76, 77-112-152]. Lactobacilli species typically make up the majority of the vaginal microbiota, ranging from 70 to 90 % [5] and *Lactobacillus (L.) crispatus*, gasseri, iners, jensenii, reuteri, rhamnosus, and fermentum appear to be the most beneficial for vaginal health [74, 76, 77-112-152]. These bacteria produce lactic acid in an estrogen-rich vaginal epithelial cells through the process of glycolysis, which helps to maintain a slightly acidic pH (3.5-4.5) in the vagina, creating an environment hostile to harmful bacteria and other microorganisms[70-76-152]. Additionally, *Lactobacilli* produce hydrogen peroxide (H_2O_2), bacteriocins, and biosurfactants that have antimicrobial activity against other bacterial species. Wu et al., (2024) [76] reported that factors such as antibiotics, hormonal changes, and sexual activity can disrupt this delicate balance, leading to an overgrowth of harmful bacteria and the development of conditions such as bacterial vaginosis (BV) and fungal infections [74, 76, 77-112-152]. There are several other studies have confirmed the effectiveness of oral probiotics in colonizing the vaginal environment, possibly by migration of some Lactobacillus strains from the rectal mucosa and perianal area directly to the vagina [76-152]. These beneficial probiotics establish themselves and compete with harmful microorganisms for space and resources and help maintain a balanced and health vaginal microbiome[76-152].

Bacterial vaginosis is a common vaginal infection with a prevalence of around 10e20 % in most of the Asia countries [74, 76, 77-112]. It is caused by an imbalance in the vaginal microbiota and associated with important adverse health outcomes such as increased risk of sexually transmitted infections (STIs), infertility, pelvic inflammatory disease, and pregnancy complications [74, 76, 77-112-152]. The current treatment approach for BV typically involves antibiotic therapy. However, probiotics containing L. crispatus, L. jensenii, and L. gasseri have been found to be effective in treating BV[76-152].

Vaginal candidiasis is a common fungal infection involving vagna. It affects about 75 % of women at least once in life and is characterized by leukorrhea, intense pruritus, vulvar hyperemia, dysuria, and dyspareunia [74, 76, 77-112-152]. The current treatment approach typically involves antifungal therapy. *L. gasseri* and *L. crispatus* have been found to be effective in inhibiting the growth of *Candida albicans* [76-153].

Infertility is a widespread global health concern that impacts millions of individuals in their reproductive years[74, 76, 77-112-152]. Current data indicates that approximately one out of every six individuals worldwide will encounter infertility during their lifetime [76-152]. Various factors have been identified as potential causes of infertility, with age standing out as one of the most signific determinants of fertility decline while dysbiosis of vaginal microbiota is reported associated with female infertility [74, 76, 77-112-152]. Research has demonstrated that a high-Lactobacillus vaginal microbiota is correlated with a reduced risk of female infertility [76]. Given the ability of probiotics to promote a high-Lactobacillus vaginal microbiota, it is reasonable to hypothesize that probiotics could have a beneficial effect on women experiencing infertility[74, 76, 77-112-152].

Genitourinary syndrome of menopause mostly occurs when the urogenital tissues become thin, dry, and inflamed due to decreased levels of estrogen, typically in menopausal women[74, 76, 77-112-153]. Symptoms such as vaginal

dryness, burning, itching, pain during intercourse, and urinary problems can lead to discomfort, embarrassment, and decreased sexual function. These symptoms can also impact women's psychological well-being and lead to anxiety, depression, and a decreased sense of well-being. The treatment approach typically involves hormonal therapy or lifestyle modifications. Several clinical studies have shown that certain strains of probiotics (*L. acidophilus*) taken together with ultra-low-dose (0.03 mg) vaginal estriol (E3) can improve GSM symptoms. Such improvement may be achieved through restoring the thickness and elasticity of the vaginal tissues and the natural acidity of the vagina, which can help to prevent the overgrowth of harmful bacteria and yeast[74, 76, 77-112-152].

Chlamydia infection is caused by the bacterium Chlamydia trachomatis while Gonorrhea is caused by the bacterium *Neisseria gonorrhoeae* and both pathogens can infect men and women[74, 76, 77-112-152]. These diseases are common STIs worldwide and often asymptomatic. The estimated global prevalence in women is approximately 3.8 % for chlamydia and 0.9 % for gonorrhea [74, 76, 77-112-152].

Polycystic ovary syndrome is characterized by an excess production of androgens by the ovaries, leading to a wide range of symptoms including chronic anovulation, cysts within the ovaries, acne, hirsutism, insulin resistance, and weight gain. It is also a common cause of infertility in women. The pathogenesis of PCOS is not known because it is a complex multi-genetic disorder. Globally, the estimated mean prevalence of PCOS is around 21 %[74, 76, 77-112-152].

Human papillomavirus (HPV) infection is the most common viral infection of the female reproductive tract, with an estimated 79 % of sexually active women will contract at least one HPV infection at some point in their lives [74, 76, 77-112]. However, not all women with HPV will develop symptoms or health problems related to the infection. Most HPV infections are transient and clear up spontaneously within 1e2 years without causing any clinical symptoms or complications. However, some infections can persist and progress to precancerous lesions and eventually to invasive cervical cancer over a period of several years to decades[74, 76, 77-112-152].

Endometriosis is a chronic inflammatory condition that can cause painful menstruation and infertility, that affect about 10 % of women during their reproductive age. The pathogenesis of endometriosis is still not fully defined and is considered as a multifactorial process. Retrograde menstruation and coelomic metaplasia are currently the most recognized pathogenetic hypotheses[74, 76, 77-112-152-152].

Preterm labor, also known as premature labor, defined as the onset of regular uterine contractions and cervical changes before the 37th week of pregnancy. Most of preterm labors are associated with maternal infection[74, 76, 77-112-152]. Other causes include cervical incompetence, multiple gestation, maternal stress, and certain medical conditions such as diabetes and hypertension. However, the real cause for many cases of preterm labor is still undetermined. Preterm birth causes 60 % to 80 % of neonatal deaths while survivors can experience life-long complications [76]. Due to the antiinflammatory properties exhibited by probiotics in the genital tract, numerous studies have explored their potential role in preventing preterm labor. However, the evidence is limited and inconclusive[74, 76, 77-112-152].

Gestational diabetes mellitus (GDM) is a type of diabetes that develops during the second or third trimester of pregnancy and typically subsides after delivery. Genetics and lifestyle factors such as obesity, physical inactivity, and unhealthy diet can increase the risk of developing GDM [74, 76, 77-112-152].

There are several reasons for the possible occurrence of anemia during pregnancy. One of the most common causes is an increase in blood volume during pregnancy, which can lead to a dilutional effect and a decrease in to decrease the concentration of red blood cells[74, 76, 77-112-152]. Additionally, if the mother's iron stores are insufficient for fulfill the increased demand for iron during pregnancy to support fetal growth and development, anemia may occur. They concluded that intake of probiotics from early pregnancy was safe, attenuated the loss of iron stores and improved iron status in healthy pregnant women[74, 76, 77-112-152].

Maternal GBS colonization refers to the presence of *Streptococcus agalactiae* in the mother's body during pregnancy. It is estimated that 10e30 % of pregnant women carry GBS in their vaginal or rectal area at any given time. In most cases, GBS does not cause any symptoms or harm to the mother, but it can be passed to the newborn during delivery and cause serious infections[74, 76, 77-112-152]. A preclinical study found that probiotic was able to inhibit the growth of GBS in vitro, suggesting a potential role in preventing GBS colonization[74, 76, 77-112-152].

Habitual abortion, also known as recurrent pregnancy loss, is defined as the occurrence of three or more consecutive pregnancy losses before the 20th week of gestation. There were various causes been identified, including genetics, anatomical, endocrine, immunological, and placental anomalies[74, 76, 77-112-152]. However, in many cases, the exact

cause remains unknown. One of the causes of habitual abortion is the alteration of spermatozoa antigenicity such as human leukocyte antigen (HLA) [74, 76, 77-112-152]. Studies have shown that sperm HLA expression is reduced in males whose partners have a history of recurrent miscarriage, and such reduced HLA expression has been associated with a decreased risk of developing anti-paternal cytotoxic antibodies in their female partners[74, 76, 77-112]. According to one of the study concluded that probiotics supplement may be beneficial for couples suffering from habitual abortion with an immunologic cause[74, 76, 77-112-152]. Till now, only one pilot clinical trial investigating the potential role of probiotics in habitual abortion with 20 women who had experienced 3 miscarriages within the first 12 weeks of pregnancy[74, 76, 77-112]. *Lactobacillus salivarius* was administrated daily to these recruited women for a maximum of 6 months. At the end of study, 15 of them had subsequent full-term pregnancies while only one had an abortion [74, 76, 77-112-152]. This promising result encourages investigators to conduct a larger, more thorough study such as RCT to confirm the findings.

Lactational mastitis usually occurs in breastfeeding women. This condition typically starts in the first few months after giving birth, but also possible at any time during lactation[74, 76, 77-112-152]. It can lead to decreased breastfeeding rates, which then may lead to several consequences such as increased risk of infant morbidity, negative impact on maternal health, and increased healthcare cost[74, 76, 77-112-152]. There is some evidence that probiotics may participate in preventing and treating mastitis in breastfeeding women. A review of clinical studies published in 2022 included 6 RCTs indicated that probiotic supplementation during pregnancy may reduce the risk of mastitis (OR: 0.49, 95 % CI 0.35e0.69) and significantly reduced the bacteria counts in the milk of mastitis patients [74, 76, 77-112-152].

The application of probiotics is a safe, effective, and natural way to support women's health[74, 76, 77-112]. Incorporating probiotics into a woman's daily routine, either through supplementation or dietary sources, may offer numerous benefits for gynecologic and obstetric health, including preventing STIs and treating vaginal infections, improving metabolic and hormonal profiles in PCOS women, reducing the risk of obstetrics complications such as GDM, maternal GBS colonization, obstetric anemia, and postpartum mastitis, alleviating menstrual pain and endometriosis-related symptoms, and potentially even reducing the risk of certain gynecologic cancers. These benefits are likely due to the ability of probiotics to restore and maintain a healthy vaginal microbiome, as well as to modulate the immune system[74, 76, 77-112-152].

They are a blend of live microorganisms with benefits to the body—beyond the health of the digestive tract [74, 76, 77-112]. For example, the skin, mouth, penis and vagina have unique microbiomes of their own[3-9]. While commercial probiotics catering to all of these microbiomes do not yet exist, vaginal probiotics do [74, 76, 77-112-152]. They dominate the shelves of drugstores and supermarkets with the notion that "vaginal" probiotics can boost vaginal health, similar to how "gut" probiotics can improve gut health [74, 76, 77-112]. **Vaginal probiotics** promise to seed the vagina with "good" microbes that keep the pH and odor in check and support the health of the vaginal and urinary tract [74, 76, 77-112-152]. However, most of the claims lack scientific evidence to support them [74, 76, 77-112-152]. Some small-scale clinical trials have tested the use of vaginal microbes (probiotics) to treat bacterial, yeast and sexually transmitted infections, but none have demonstrated effectiveness that supports FDA clearance and large-scale marketability The vaginal microbiome is not static [74, 76, 77-112-152]. Microbial communities shift with different seasons of life, including birth, pre-puberty, puberty and menopause [74, 76, 77-112-152]. Genetics, geography, ethnicity and lifestyle factors also impact vaginal microbiome populations. Defining a disease-free vaginal state is, therefore, complicated; but, in general, the vaginal microbiota can be classified into 5 community state types (CSTs) [74, 76, 77-112-152]. These CSTs can be thought of as the 5 most likely combinations to make up the vaginal microbiota in women of reproductive age. *Lactobacilli* species occupy about 70% of the healthy vaginal microbiome during reproductive years, but *Lactobacilli* found in the vagina are not the same as the ones that reside in the gut [74, 76, 77-112-152]. In fact, although 250 types of *Lactobacilli* are known in nature, 4 out of 5 CSTs are dominated by *Lactobacilli* species: *L. crispatus, L. gasseri, L. iners* and *L. jensenii*, which are generally known for their protective qualities[74, 76, 77-112-152]. For instance, these organisms convert sugars in the vaginal walls to lactic acid, generating a slightly acidic environment that is hostile to incoming pathogens. Additionally, they produce antimicrobial peptides, such as bacteriocin, to kill competing microbes, and can even physically take up space to keep pathogens from establishing themselves [74, 76, 77-112-152]. The vaginal microbiota (VMB) plays a crucial role in women's health from puberty to menopause [74, 76, 77-112-152]. Traditional studies have focused on the microorganisms present within the vaginal environment and their roles in disease onset. However, the dynamic relationship between the VMB and its host remains underexplored.

Probiotics, especially certain *Lactobacillus* species, can benefit vaginal health by maintaining a balanced vaginal microbiome, preventing infections, and supporting an acidic environment. These beneficial bacteria can help to reduce the occurrence of bacterial vaginosis (BV) and other vaginal infections [74, 76, 77-112-152]. Consuming probiotics has become a popular way to improve digestive health. Probiotics are healthy bacteria strains found naturally in some foods

and in nutritional supplements [74, 76, 77-112-153]. More recently, health experts have begun to consider the potential benefits of probiotics on vaginal health[3-9]. *Lactobacillus acidophilus* is the most-researched strain of probiotic when it comes to establishing and maintaining a healthy vaginal balance [74, 76, 77-112-152]. Two other important strains include *Lactobacillus rhamnosus* and *Lactobacillus reuteri*[74, 76, 77-112]. Experts believe these strains helps to maintain vaginal balance by sticking to vaginal surfaces and making it more challenging for harmful bacteria to grow. Lactobacillus may also adhere directly to harmful bacteria, killing them and preventing them from spreading[74, 76, 77-112-152] There are many microbial colonization in the vagina of healthy women, among which Lactobacillus plays a major role (95%)[74, 76, 77-112-152].

Vaginal dysbiosis and related infections are common, often causing discomfort and leading to medical consultations. Conventional treatments with antibiotics and antifungal drugs are not always effective and can disrupt beneficial lactobacilli, potentially leading to recurrent BV and VC[74, 76, 77-112-152]. Therefore, more effective and natural solutions are needed to balance the VMB and maintain a healthy vaginal environment. Probiotics, particularly lactobacilli, have emerged as promising candidates for maintaining or restoring a healthy VMB post-antibiotic treatment [74, 76, 77-112]. Systematic reviews and meta-analyses suggested that probiotics may help mitigate BV and VC, enhancing the quality of life for affected women [74, 76, 77-112-152]. Probiotics support the balance of the vaginal microbiota, promoting the growth of beneficial bacteria, preventing harmful microorganisms' overgrowth, and strengthening the immune system[74, 76, 77-112-152].

Probiotics have been widely used in the treatment of intestinal diseases, but the effect of probiotics on female reproductive tract health is still controversial Lactobacillus is the most abundant microorganism in the vagina, which is related to the vaginal mucosal barrier[74, 76, 77-112-152]. *Lactobacillus* adheres to the vaginal epithelium and can competitively antagonize the colonization of pathogens[74, 76, 77-112-152]. The factors produced by Lactobacillus, such as bacteriocin and hydrogen peroxide (H_2O_2), can inhibit the growth of pathogenic microorganisms and maintain the low pH environment of the vagina[74, 76, 77-112-152]. Probiotics play an important role in maintaining the stability of vaginal microenvironment, improving immune defense and blocking the progression of cervical cancer. There is an association between a highly diverse vaginal microbiota and female reproductive tract health [74, 76, 77-112-152]. Probiotics play an important role in maintaining the health of the female reproductive tract, alleviating gynecological diseases, and enhancing the local immunity of the vagina[3-9]. The use of probiotics or VMT intervention has a certain effect on preventing the progression of CIN, treating BV, and relieving symptoms related to senile vaginitis[74, 76, 77-112]. The development of 16SrRNA sequencing technology can help to identify microbial markers and carry out personalized prevention and treatment of diseases[74, 76, 77-112]. At present, the mechanism of action of probiotics in cervical cancer is not fully understood[74, 76, 77-112-152].

Very recently Patki et al., (2025) [113] reported that various gynecological conditions, including bacterial vaginosis, urinary tract infection, genitourinary syndrome of menopause, polycystic ovarian syndrome, and vulvovaginal candidiasis, impose a significant global burden, including among the Indian population [113-152]. This expert opinion emphasizes the importance of oral probiotic supplementation in managing these conditions. A physical meeting with 14 experts was conducted on June 29-30, 2024, during which they highlighted that probiotics, particularly Lactobacillus species, have beneficial effects on restoring and maintaining healthy vaginal microbiota[113-152]. Probiotics also promote vaginal health and aid in treating conditions such as bacterial vaginosis, vulvovaginal candidiasis, polycystic ovarian syndrome, and genitourinary syndrome of menopause [113-152].

Probiotics are live microorganisms that provide health benefits beyond basic nutrition when consumed in adequate amounts [1- 113-153]. The most common probiotic bacteria include *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus reuteri, Lactobacillus plantarum, L. casei GG, Bifidobacterium brevis, Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium animalis,* and *Streptococcus thermophilus*. Some yeast strains, such as Saccharomyces boulardii, also exhibit probiotic properties [1-113-153]. Probiotics promote health through several mechanisms: enhancing the epithelial barrier of the gut to reduce intestinal permeability and prevent pathogen invasion; producing antimicrobial substances such as bacteriocins, organic acids, and hydrogen peroxide to inhibit the growth of harmful bacteria; and competing with pathogenic bacteria for adhesion sites on the intestinal mucosa to prevent their colonization[113]. Additionally, probiotics modulate the host's immune response by stimulating the production of anti-inflammatory cytokines and suppressing pro-inflammatory cytokines, thus balancing the immune system and reducing inflammation[113]. These actions contribute to maintaining a healthy gut microbiota and improving overall health [113-152].

The effectiveness of probiotics and synbiotics in managing polycystic ovarian syndrome is well-supported by research[113-152]. Studies indicate that supplementation with probiotics or synbiotics can improve hormonal imbalances, reduce inflammation, and address lipid metabolism issues associated with polycystic ovarian

syndrome[113-152]. Additionally, research shows that these supplements may help manage weight, lower BMI, improve insulin levels, and reduce HOMA-IR, potentially playing a role in protecting fertility [113-150]. Probiotics have been shown to alleviate polycystic ovarian syndrome symptoms by modulating gut microbiota. They increase levels of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*, thereby restoring microbiota balance[113-153].

Overall, the administration of intravaginal estrogen and/or probiotics in pre-menopausal ACB women is feasible, safe, and well tolerated[3-9-152]. Although *Lactobacilli* dominate a healthy vaginal microbiota, another bacterial species with the ability to release lactic acid, *Bifidobacterium,* seems to play a protective role in the vagina as well [74, 76, 77-112-150]. These bacteria can exist side-by-side with *Lactobacilli*. But reports find *Bifidobacterium*-dominant microbiome in 5-10% of healthy reproductive-aged women, suggesting they could potentially correspond to a new CST [74, 76, 77-112-150]. Overall, *Lactobacilli* and *Bifidobacteria* are indicators of a healthy vaginal microbiome, but there are limited data to support whether using probiotics to replenish either of these species can improve vaginal health and alleviate infections [74, 76, 77-112-152]. Overall, *Lactobacilli* and *Bifidobacteria* are indicators of a healthy vaginal microbiome, but there are limited data to support whether using probiotics to replenish either of these species can improve vaginal health and alleviate infections[74, 76, 77-112-152]. The application of probiotics is a safe, effective, and natural way to support women's health[74, 76, 77-112-152]. Incorporating probiotics into a woman's daily routine, either through supplementation or dietary sources, may offer numerous benefits for gynecologic and obstetric health, including preventing STIs and treating vaginal infections, improving metabolic and hormonal profiles in PCOS women, reducing the risk of obstetrics complications such as GDM, maternal GBS colonization, obstetric anemia, and postpartum mastitis, alleviating menstrual pain and endometriosis-related symptoms, and potentially even reducing the risk of certain gynecologic cancers [74, 76, 77-112-152]. These benefits are likely due to the ability of probiotics to restore and maintain a healthy vaginal microbiome, as well as to modulate the immune system [74, 76, 77-112]. Further research is required to fully understand the action mechanisms of probiotic, although current evidence strongly supports their use in promoting women's health [74, 76, 77-112-152]. In gynecologic conditions, probiotics showed potential in preventing and managing bacterial vaginosis, vulvovaginal candidiasis, and sexually transmitted infections. During pregnancy, probiotics may be helpful in reducing the risk of gestational diabetes, maternal group B streptococcal colonization, obstetric anemia, and postpartum mastitis. Overall, *Lactobacilli* and *Bifidobacteria* are indicators of a healthy vaginal microbiome, but there are limited data to support whether using probiotics to replenish either of these species can improve vaginal health and alleviate infections[74, 76, 77-112-152]. Further research is required to fully understand the action mechanisms of probiotic.

Lactobacillus species, especially among probiotics, play an important role in the treatment and prevention of various gynecological disorders [74, 76, 77-113-152]. They help restore and maintain the natural vaginal microbiota balance, supporting vaginal health and managing conditions such as bacterial vaginosis, vulvovaginal candidiasis, and polycystic ovarian syndrome[74, 76, 77-112-152]. Evidence suggests that probiotics may alleviate symptoms of genitourinary syndrome of menopause in postmenopausal women and promote urinary tract health, preventing recurrent urinary tract infections[74, 76, 77-112-152]. While clinical recommendations are limited by available evidence, probiotic interventions appear to be an effective alternative or adjunctive treatment option for urogenital infections [74, 76, 77-112-152]. Probiotics should be considered advantageous in bacterial vaginosis treatment, particularly when administered alongside antibiotics, to enhance treatment efficacy and prevent recurrence [74, 76, 77-112]. Lactobacillus strains such as *L. rhamnosus* GR-1 and *L. reuteri* have been recommended for urinary tract infection prevention and maintaining urinary tract health, also reducing the risk of antibiotic resistance[74, 76, 77-112-152]. Probiotic supplementation has also been shown to improve pregnancy rates in women with polycystic ovarian syndrome. Overall, probiotics represent a promising treatment option for a range of gynecological conditions [74, 76, 77-112-152].

7. Conclusion

Probiotics are living microorganisms that have the potential to be beneficial to host organisms when administered at the correct dosage. Humans have benefitted from microorganisms in food in various forms throughout history. The benefits of including certain live microbes in food were first indirectly observed in the health effects of fermented foods, though the cause would have been almost certainly unknown at the time. While most well-characterized probiotic microbes are bacteria such as *Bifidobacteria* and *Lactobacillus, and* certain yeasts have been shown to have health benefits across various studies. The most common yeast with proposed probiotic effects is *Saccharomyces boulardii.* Also known as *Saccharomyces cerevisiae* var. *boulardii* or *Saccharomyces cerevisiae* Hansen CBS 5926, The counter preparations of this yeast are typically recommended for the treatment of acute gastrointestinal diseases such as rotoviral and bacterial diarrhea. Most probiotics are lactic acid bacteria (LAB) with the majority belonging to the genera *Lactobacillus, Enterococcus and Bifidobacterium*. However, to date only a few yeast strains have been identified as probiotics. *Sacchromyces cerevisiae* and *S. boulardii* are the only commercial probiotic yeast species currently

available for human consumption. The vaginal microbiota (VMB) plays a crucial role in women's health from puberty to menopause. Traditional studies have focused on the microorganisms present within the vaginal environment and their roles in disease onset. However, the dynamic relationship between the VMB and its host remains underexplored. Probiotics and prebiotics also have some broader implications for vaginal health, including their role in minimizing the incidence of premature birth, optimizing fertility, managing menopausal symptoms, and preventing vaginal infections. Probiotic supplementation has also been shown to improve pregnancy rates in women with polycystic ovarian syndrome. Overall, probiotics represent a promising treatment option for a range of gynecological conditions.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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