


Spontaneously Fermented Cereal Based Products: An Ancient Health Promoting Formulae for Consumption of Probiotic Lactic Acid Bacteria

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Abstract: The adverse health effects of dairy-based products like lactose intolerance, cholesterol content, cow milk allergy, and religious sentiment are a few concerns that lead to an interest in non-dairy fermented foods and beverages prepared using cereal, legumes, and millet. Cereal fermentation by Lactic Acid Bacteria (LAB) is an ancient health-promoting technique with proven health benefits like controlling gastrointestinal disorders, managing cardiovascular diseases, cholesterol reduction, anticarcinogenic activity, immunomodulation, production of antimicrobial agents, and antitumor activities apart from providing nutrition. Several studies have explored probiotic LAB from fermented dairy products. However, limited studies are available, especially in the Indian context, that demonstrate the importance of Indian cereal-based fermented products as carriers and sources of potential probiotic strains. It is, therefore, vital to bridging the knowledge gap underlying the opportunities hidden in Indian cereal-based functional fermented products, their exploitation as a source of probiotics, and potential health benefits harbored for the global population. Therefore, this paper reviews recent research on different spontaneously fermented cereal-based foods and beverages of the Indian subcontinent, which are a potential source of probiotics, and summarizes the advances made in identifying and characterizing potential probiotic attributes of LAB strains from these products. The significance of LAB in cereal-based products, evaluation parameters of probiotic strains, experimental probiotic products, commercial potential, challenges, and future perspectives are also discussed.

Keywords: cereals; fermented foods; beverages; lactic acid bacteria; probiotic potential.

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1. Introduction

A large quantity of cereals worldwide is used for obtaining fermented foods using microbes. Since time immemorial, fermentation has been used to preserve foods and obtain health benefits. The importance of fermentation is evident because various fermented foods and beverages are part of most communities' culinary and cultural heritage [1–3]. There is no information about the first use of intentional fermentation, but archeological findings indicated bread and cheese making around 6000-7000 BC [4]. Spontaneous or traditional fermentation is primarily carried out by microbes, especially Lactic Acid Bacteria (LAB) and yeast [5,6]. It is a natural and economical way to enhance sensory quality, extend shelf life, improve nutritional value, digestibility, and palatability, and destroy undesirable spoilage-causing and pathogenic microbes [6,7]. Spontaneously fermented cereal-based foods are a significant part of the daily diet and calorie intake in Asian and African countries. Some of them are used as light meals or breakfast and weaning food for children [1,8]. Several cereal-based fermented products prepared and developed by human beings in different contexts testify to cultural diversity [9].

Cereals and their constituents have gained popularity as functional foods and nutraceuticals in recent years due to their ability to deliver critical nutrients such as dietary fiber, proteins, energy, minerals, vitamins, and antioxidants [5]. Rice, wheat, corn, rye, barley, sorghum, millets (Pearl, Kodo, barnyard, foxtail, proso, little millet), pseudo-cereals (buckwheat, quinoa, and amaranth) are the most common cereals consumed by humans. These cereals are high in phytochemicals, antioxidant-rich in dietary fiber, minerals (zinc, iron, and magnesium), vitamins, and a dietary energy source due to their carbohydrate and protein content [5,10]. Cereals can also be used as fermentable substrates for probiotic microorganism growth [5,10,11] as they are a staple food worldwide. Various technologies are used to transform cereals into foods and beverages, but fermentation is likely the best method for improving nutritional and sensory properties and shelf life. Before consumption, a large portion of the world's cereal production is fermented. Various types of food products, including fermented foods and beverages manufactured using these cereals. Using these cereals to produce functional fermented foods is an area of great interest to feed a growing population and overcome food and nutritional security caused by environmental degradation. Various scientific reports demonstrated the positive effect of consuming these products against various diseases like cardiovascular problems, diabetes, and some types of cancer [12–16]. The improved shelf-life, acceptability, and digestibility resulting from fermentation and the enhancement of organoleptic properties and texture are important reasons for this [17]. Although bacteria, yeasts, and fungi are necessary for fermentation, LAB is the most common microflora involved in the fermentation of these products. These LAB are generally considered safe (GRAS) for human consumption [18].

LAB associated with cereal fermentation has been isolated from various food products that exert various beneficial attributes in these products, such as the production of compounds like organic acids, antimicrobial substances, bioactive peptides, and characteristic flavoring compounds. Several studies have been done to isolate these LAB and establish their beneficial attributes through *in vitro* and *in vivo* methods. Many of these LAB are considered probiotics. Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit" [19]. *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Bifidobacterium*, and

Enterococcus are the major genus considered probiotics, but Lactobacillus and Bifidobacterium have the credible and longest safe history for human consumption as probiotic [20]. The probiotic LAB inhibits the pathogens and produces antimicrobial substances, including bacteriocin and other potential functional bioactive compounds [21–25]. The probiotics are shown to manage gastrointestinal disorders and enhanced beneficial activities such as anticholesteremic, immunomodulatory, antidiabetic, anti-inflammatory, anti-obesity, and antioxidant [10,23,26–29], mental health [30], balances the intestinal microflora [31]. LAB is reported to prevent and treat several diseases, such as lactose intolerance, antibiotic-induced diarrhea, infantile diarrhea, travelers' diarrhea, constipation, colon cancer, hypercholesterolemia, and vaginitis [25,32–34].

Cereals can also provide prebiotic and whole-grain benefits to consumers [26,35–37]. According to the most recent and revised prebiotic definition, prebiotics is now defined as "a substrate that is selectively exploited by host microorganisms giving a health advantage" rather than non-digestible oligosaccharides of food origin [38]. Therefore, in addition to carbohydrates, other substances like fatty acids, peptides, phenolics, and inorganic micronutrients may have prebiotic effects [26]. A dietary product that contains both a probiotic and a prebiotic is referred to as a "synbiotic" [25]. The benefits of this combination have been demonstrated to outweigh the benefits of probiotics and prebiotics alone. If a probiotic strain uses the prebiotic carbohydrate, its development and proliferation in the gut will be boosted selectively [39].

Although most probiotic meals are now dairy-based, there is rising interest in isolating probiotic strains from various dietary sources, such as fruits, vegetables, legumes, and cereal foods. Recently, functional food research has progressed toward developing dietary supplements, with the introduction of probiotics and prebiotics, which may impact gut bacteria composition and activities. However, despite the growing interest of researchers and the food industry in probiotic LAB strains, there is limited use as probiotics, especially in India. Very limited probiotic products can fulfill the strict international regulatory norms, and most of them fail in the clinical trial stage [40]. Nevertheless, the rural population has been using cereal-based fermented products with probiotic microbes and getting health benefits since the dawn of civilization in the Indian subcontinent. At the same time, identifying and characterizing probiotic strains from various habitats may significantly enhance the value of such bacteria in the future. Future research and advancements in functional foods may involve diversification other than dairy products. Therefore, this review paper emphasizes the nutritional and health aspects of cereals used for fermentation, diversity, and associated microflora of Indian cereal-based fermented products and beverages, the probiotic potential of isolated lactic acid bacteria, and the health benefits of consumption. Furthermore, the commercial potential of probiotics, experimental cereal-based probiotic products, and challenges and recommendations for future perspectives are also discussed.

2. Food Fermentation by Lactic Acid Bacteria

LAB has been used since 6000 BC, with mentions of fermentation in dairy and meat dating back to 1500 BC and plants dating back to 300 BC [41]. These microbes are very important for manufacturing fermented dairy and food products. LAB has a long history of safe use, particularly in the dairy industry, and is used extensively in fermented dairy products

[24,42]. The major end product of carbohydrate utilization by LAB is lactic acid [10,12,43–45]. LABs are widespread microbes in almost any habitat and product, including plants, fermented foods, and human mucosal surfaces. Due to their distinct flavor-forming and metabolic activities, fermented items are the primary source of LAB isolation. LAB are widely employed in biotechnology, culinary, and pharmaceutical products due to their diverse metabolism and ability to manufacture a wide range of valuable metabolites in addition to lactic acid [46].

Lactic acid bacteria have been isolated from a variety of sources like raw matrices (grains, crops, plant surfaces, and silages), fermented food products prepared by fermentation (milk, cereals, fruits, and vegetables), gastrointestinal and vaginal tracts, to mention a few [44,47–49]. Because of the wide variety and number of applications of LAB, it is required to link genetic proof to their fundamental properties to fully utilize their metabolic applications [46,50]. The major application of LAB is inhibitory to food spoilage and pathogenic microbes, antimicrobial and antifungal agents, bacteriocin production, acting as live beneficial microbes (probiotics), and fermentation [12,21,22]. LAB are divided into two groups (homofermentative – produces lactic acid as the end product and heterofermentative – lactic acid, CO₂, and ethanol) on the basis of their sugar utilization pattern [51]. Other compounds such as hydrogen peroxide, bacteriocin, flavoring compounds, peptides, and fatty acids are also formed during these fermentations, which exert antagonistic activity against other undesirable microbes [52]. These compounds enhance these products' overall acceptability, food safety, and shelf life by inhibiting unwanted microflora [48,50]. These microbes are also reported to decrease tannin content in many high-tannin-content crops to improve iron absorption [53,54].

The emergence of the need for improved starter cultures provoked the idea of exploiting microbial diversity in traditionally unique natural systems. Each fermented product species provides a distinct environment regarding competing microorganisms, natural vegetal antagonists, and substrate accessibility, type, and concentration in various physical factors. Despite these similarities, there are significant differences in substrates and products and the types of microbes used in producing fermented foods and beverages worldwide.

3. Cereals as Food Fermentation Substrates

Cereals are staple foods and a significant part of daily dietary intake to obtain calories at affordable prices worldwide [55]. They are widely available because they are grown in 74% of the world's land area and contribute roughly half of all food produced globally [5]. Rice (*Oryza sativa*), wheat (*Triticum aestivum*), corn (*Zea mays*), rye (*Secale cereal*), barley (*Hordeum vulgare*), sorghum (*Sorghum Vulgare*), millets (Finger, pearl, barnyard, foxtail, proso, Kodo, little millet), and some pseudo-cereals (buckwheat, quinoa, and amaranth) are the most common cereals consumed by humans [5,10,16,56]. All of these cereals are high in phytochemicals, antioxidant-rich dietary fiber, minerals (zinc, iron, and magnesium), vitamins, and a source of dietary energy due to their carbohydrate and protein content (Figure 1) [5,10,57]. Whole pseudo-cereal grains like buckwheat, amaranth, and quinoa are also high in a variety of compounds like flavonoids, phenolic acids, trace elements, fatty acids, and vitamins, all of which have been shown to have beneficial effects on human health, including the prevention and reduction of a variety of degenerative diseases [58–60]. Pseudo-cereals are underutilized gluten-free crops, high in protein, and contain a wide range of crucial nutrients.

These pseudo-cereals also contain saponins, which have a variety of agricultural, pharmaceutical, and industrial applications [59,61].

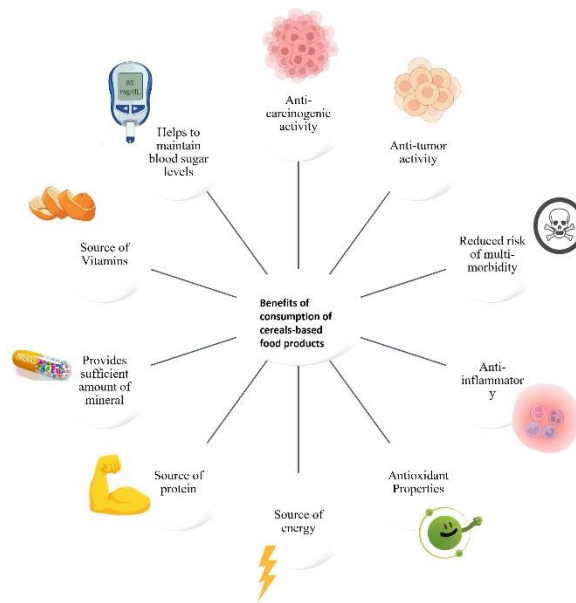


Figure 1. Benefits of cereal consumption.

3.1. Bioactive ingredients of cereals.

The plant contains several non-nutritive ingredients which show functional bioactivities and are known as phytochemicals. These ingredients are reported to exhibit various health-promoting attributes due to their antioxidative potential, antibacterial activity, and other biologically beneficial traits. These compounds can also minimize the chance of several chronic diseases [11,62,63] and are vital for developing pharmaceutical products [14,16]. The phytochemicals are found in different parts of cereal crop plants, but a major portion is reported in the kernel and bran [8]. The various phytochemicals in cereals are flavonoids, phenolic acids, steroids, alkaloids, carotenoids, tannins, saponins, anthocyanins, and other phenolics [64–66]. Consumption of whole cereal grains has been linked to having beneficial effects by lowering the chances of cancer [14,67], cardiovascular disease [15,68,69], high blood pressure [38], and diabetes [16,70,71].

4. Diversity of Indian Spontaneously Cereal Based Fermented Foods and Beverages

Several fermented foods and beverages are manufactured throughout the world using cereals, having area-specific local variations of composition and process of manufacturing [3,25,72,73]. Although India has a wide variety of fermented foods, their production and consumption are limited to a particular geographical location and specific community. The preparation of traditionally fermented cereal-based foods by LAB is one of the oldest biotechnological processes intended to extend shelf-life and sensory attributes, improve palatability, digestibility, health-promoting properties, and the nutritive value of food [1,7,74–76] in Asia and Africa [2,50]. These products are produced by spontaneous fermentation, and the quality of the finished product depends on initial counts of microbes and raw ingredients. This spontaneous fermentation is carried out using the previous batch fermented product known as back-slopping. The back-slopping process helps decrease fermentation time and

obtain a predictable quality product. This process is used for making various fermented cereal products like idli, xaj, dosa, raabadi, apong, and haria [77,78]. Generally, starter cultures are not used for the production of these products. The raw cereal materials used in manufacturing fermented cereal-based dishes or the texture of the fermented products can be used to classify them. These foods are prepared at the domestic level or industrial scale using raw grains/flour or malted grains for fermentation [78].

Lactic acid bacteria (LAB) are found to be associated with several fermented foods and beverages [2,79]. The cereal-based fermented foods commonly contain LAB species of *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Micrococcus*, and *Bacillus*, although *Lactobacillus* species are the predominant microbes [3,23,44]. Other than bacteria, some fungal genera are also reported to be associated with fermented products, such as *Saccharomyces*, *Cladosporium*, *Aspergillus*, *Trichothecium*, *Paecilomyces*, *Fusarium*, and *Penicillium* [80–82]. However, *Saccharomyces* are commonly used to produce alcoholic products [83,84]. The optimum growth of fermenting micro-flora depends on various factors like pH, available moisture, redox potential, temperature, osmotic stress, and food matrix composition [85]. The various types of fermented foods and beverages of the reviewed region and reported associated microflora are summarized in table 1.

Table 1. Different types of cereal-based functional foods and their microflora.

Fermented Food/Beverage	Microbes associated	Major cereal as substrate	Product Description	References
1. Cereal legume-based fermented foods				
Idli	<i>Leuconostoc mesenteroides</i> , <i>Streptococcus faecalis</i> <i>Lactobacillus delbreukii</i> , <i>Lactobacillus fermentii</i> , <i>Lactobacillus plantarum</i>	Rice, black gram	Fermented steamed food	[23,75,76,78,86–90]
Dosa	<i>L. mesenteroides</i> , <i>E. faecalis</i> , <i>Lactobacillus plantarum</i>	Rice	Thin, crisp pancake, shallow-fried, staple	
Dhokla	<i>L. fermentum</i> , <i>L. mesenteroides</i> , <i>E. faecalis</i>	Rice	Mild acidic, spongy, steamed, snack	
Selroti	<i>Leuconostoc mesenteroides</i> , <i>Enterococcus faecium</i> , <i>Pediococcus pentosaceus</i> , <i>Lactobacillus curvatus</i> and Yeasts	Rice-wheat flour-milk	Pretzel-like, deep-fried bread, staple	
Khammam	<i>Pediococcus</i> , <i>Streptococcus</i> , <i>Leuconostoc</i>	Rice	Thick fermented product	
Ambeli	<i>Leuconostoc mesenteroides</i> , <i>Streptococcus faecalis</i> , <i>Lactobacillus fermentum</i>	Rice	Thick fermented batter is fermented and cooked with rice	
Sez	<i>Saccharomyces sp.</i> , <i>Bacillus sp.</i> , <i>K. marxianus</i> , and <i>S. fibuligera</i>	Rice	Semi fermented, used as a snack	
Adai and vada	<i>Pediococcus spp</i> , <i>Streptococcus spp</i> , <i>Leuconostoc spp</i>	Rice	Breakfast or Snack foods	
Bhattejaanr	<i>Hansenula anomala</i> , <i>Mucro rouxianus</i>	Rice	Sweet and sour alcoholic paste	
Anarshe	Lactic acid bacteria and yeasts like <i>Hansenula anomala</i> and <i>Mucor rouxianus</i>	Rice	Sweetened snack food	

Fermented Food/Beverage	Microbes associated	Major cereal as substrate	Product Description	References
Uttapam	<i>Lactobacillus pentosus</i> and <i>L. plantarum</i> sp.	Rice	Thickened white color, non- crispy	
Sour rice	<i>Lb. casei</i> , <i>Lb. bulgaricus</i> , <i>Pediococcus. acidilactici</i> , <i>Streptococcus thermophilus</i> , <i>Weissella</i> spp, and <i>Saccharomyces</i> spp	Rice	Cooked rice soaked and fermented in water	
Balam	<i>Saccharomycops</i> spp., <i>Candida</i> spp., <i>Mucor</i> spp., <i>Rhizopus stolonifer</i> var. <i>lyococcus</i> , <i>Rhizopus chinensis</i> , <i>Lb. bifermentans</i> , and <i>Lb. brevis</i>	Wheat flour	Dried balls used to prepare fermented alcoholic beverages	[78,89,91–98]
Bhataru or Bhatooru	<i>Saccharomyces cerevisiae</i> , and <i>Lactobacillus</i> spp., <i>Leuconostoc</i> spp	Wheat or barley flour	Baked Bread, staple	
Jalebi	<i>Lactobacillus fermentum</i> , <i>Lactobacillus buchneri</i> <i>Streptococcus lactis</i> , and <i>Saccharomyces cerevisiae</i>	Refined wheat flour	Crispy sweet, doughnut-like, deep-fried snacks	
Kulcha	<i>Saccharomyces cerevisiae</i> and LAB	Wheat	Bread with a creamy yellow color	
Babru	<i>S. cerevisiae</i> , <i>Debaromyces</i> sp., <i>L. plantarum</i> , <i>L. lactis</i> , Gram positive bacillus	Wheat or rice	Pancake; breakfast, snack	
Marcha	<i>Enterococcus</i> spp., <i>Pediococcus</i> spp, <i>Leuconostoc</i> spp, <i>Weissella cibaria</i> , <i>Saccharomyces</i> spp., <i>Wickerhamomyces</i> spp., <i>Kluyveromyces</i> spp.	Rice or wheat	Dry, mixed starter to ferment alcoholic beverages	
Chilra	<i>S. cerevisiae</i> , <i>Debaromyces hansenii</i> , and <i>Schizosaccharomyces</i> sp.	Wheat	Dosa like fermented staple food	
Kinema	<i>Bacillus subtilis</i> , <i>Escherichia facecium</i> , <i>Geotrichum candidum</i> , <i>Candida</i> spp.,	Soybeans	Alkaline, sticky; curry	
Wari	<i>Candida</i> spp., <i>Saccharomyces</i> spp, <i>L. bulgaricus</i> <i>S. thermophilus</i>	Blackgram and Soybean	Ball-like, brittle, side dish	
Tungrymbai	<i>Bacillus</i> spp., <i>Enterococcus</i> spp., <i>Lactobacillus brevis</i> , and yeasts like <i>Saccharomyces cerevisiae</i> , <i>Debaryomyces hansenii</i> , and <i>Pichia burtonii</i>	Soybean	Alkaline, sticky, curry, soup	
Bekang or Bekang-um	<i>Bacillus</i> spp., <i>Enterococcus</i> spp., <i>Saccharomyces cerevisiae</i> , <i>Debaryomyces hansenii</i> , and <i>Pichia burtonii</i>	Soybean	Alkaline in nature, paste, and curry-like consistency	
Bhalla	<i>Bacillus subtilis</i> , Lactic acid bacteria, yeasts	Black gram	Mild acidic, side dish	
Papadam	<i>Saccharomyces</i> spp, and <i>Candida krusei</i>	Black gram	Deep-fried dough of black gram bean flour	

Fermented Food/Beverage	Microbes associated	Major cereal as substrate	Product Description	References
Puda or pudla	LAB, <i>Yeasts</i>	Bengal gram, mung beans, wheat	Pancake-like bread	
2. Cereal legume-based fermented beverages				
Apong	<i>S. cerevisiae</i> , <i>Hanseniaspora</i> sp., <i>Kloeckera</i> sp., <i>Pischia</i> sp. and <i>Candida</i> sp.	Rice	clean and milky liquor	[78,90,99–105]
Koozhu	<i>W. paramesenteroides</i> , <i>Lb. fermentum</i>	rice Ragi, Sorghum, and millets	fermented porridge-like drink	
Rabadi or raab	<i>Lb. plantarum</i> , other LAB	Wheat, barley, pearl millet, or maize	Slightly acidic and viscous product	
Pazhaiya soru	<i>Enterococcus faecalis</i> , <i>Pediococcus acidilactici</i>	Rice	Consumed with buttermilk	
Haria	<i>Saccharomyces cerevisiae</i> , <i>Zygosaccharomyces cidri</i> , <i>Pichia</i> , <i>Candida</i> spp., <i>Lb. fermentum</i> , <i>Bifidobacterium</i> spp	Rice	Alcoholic beverage	
Bhaati jaanr	<i>Hansenulaanomala</i> , <i>Rhizopus</i> spp., <i>Pichia</i> spp., <i>Pediococcus</i> and <i>L. bif fermentous</i>	Rice	Thick paste, consumed as a beverage	
Zutho	<i>Saccharomyces cerevisiae</i>	Rice	Alcoholic sweet beverage	
Zu/Judima	<i>Pediococcus pentosaceus</i> , <i>Bacillus</i> spp., <i>Debaryomyces hans enii</i> ,	Rice	Alcoholic beverage	
Xaj	Yeast spp of genera <i>Rhizopus</i> , <i>Mucor</i> , <i>Aspergillus</i> , <i>Meyerozyma</i> , <i>Wickerhamomyces</i> , <i>Saccharomyces</i> , etc. and <i>Lactobacilli</i> spp	glutinous rice	Rice wine	
Chhang	<i>Lb. plantarum</i> , <i>Lb. casei</i> , <i>Enterococcus faecium</i> , <i>Pediococcus pentosaceus</i> , <i>S. cerevisiae</i> , <i>S. fibuligera</i> , <i>Pichia kudriavzevii</i> , and <i>Candida tropicalis</i>	Rice	Alcoholic beverage	
Jou	<i>S. cerevisiae</i>	Rice	Alcoholic beverage	

5. Probiotic Potential of Isolated Lactic Acid Bacteria from Cereal Products

Manufacturing cereal-based foods and beverages are done by spontaneous and uncontrolled fermentation conditions that lead to the growth of mixed microbial communities. Although most of the microbes are LAB in this type of fermentation, there has been little information about their potential as probiotics. The process of isolation, identification, and evaluation of probiotic attributes of candidate probiotic strains linked with specific end-products is complicated due to unstandardized processing conditions and variations in the type and quality of cereal ingredients [12]. Several LAB strains associated with fermented foods have been identified as having probiotic attributes or stimulating health benefits. Therefore, foods and beverages containing sufficient live cells of such strains may exert similar health-promoting attributes in the host [106]. Besides the already available knowledge of enhancement of nutritional value and enrichment of other bioactive (i.e., vitamins, phenolics), other

important metabolites are also produced by fermenting microbes that may benefit various health conditions. The genes responsible for producing these metabolites and types depend mainly on bacterial species, strain, and food matrix composition. The probiotic microbes are selected on the basis of various criteria (Figure 2) like technological attributes (cell growth, stability, and viability in raw food substrate), sensory attributes of the product, gastrointestinal tract survivability, and functionality (cell autoaggregation, cell surface hydrophobicity, bacteriocin production, immunomodulation, antimicrobial activity, and safety) [107]. Further, *in vivo* testing involving clinical trials is needed to validate such claims; however, it would be worth mentioning that few countries (e.g., Canada, Italy) have regulatory guidelines and lists of candidate probiotic strains, whereas, in others (e.g., India), dietary guidelines include fermented foods with probiotic cultures [40,106,108].

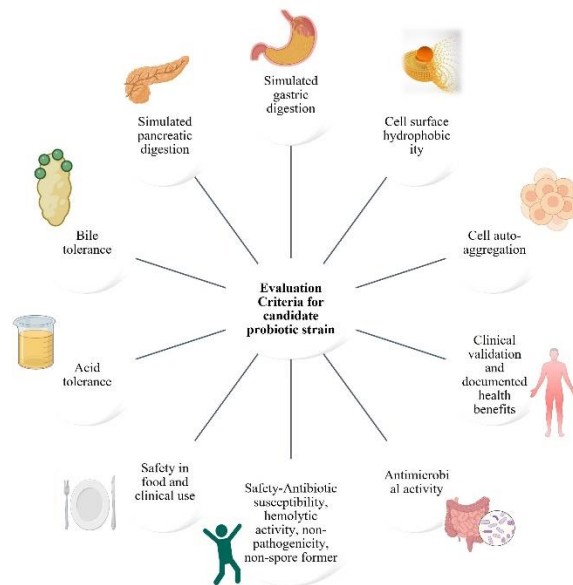


Figure 2. Evaluation criteria for the potential probiotic strains.

Due to diverse cultures and eating habits, Indian fermented foods and beverages are rich in LAB strains and have wide diversity [23,49]. Many scientific studies have been published on the isolation of several microbes from fermented milk products [23,49], but limited information is available for the isolation and evaluation of probiotic strains from cereal-based foods of south Asia, especially India, which is summarized in Table 2.

Table 2. Summary of studies done for isolation and evaluation of probiotic strains from cereal-based fermented products.

Name of Fermented Product	Potential probiotic-isolated LAB strains	Major cereal as substrate/ Sample Collection area	Reference
Idli batter	<i>Last. plantarum</i> UBLP40	Rice/South India	[9]
Sour rice	<i>Weissella confusa</i> strain GCC_19R1	Rice/Assam	[75]
Babroo	<i>Lactobacillus plantarum</i> K90, <i>L. fermentum</i> K75, and <i>L. fermentum</i> K78	Wheat/ Himachal Pradesh	[93]
Appam batter	<i>Pediococcus pentosaceus</i> NKSM1	Rice/Assam	[109]
Raabadi	<i>L. Plantarum</i> RYPR1	Pearl millet/Haryana	[100]
Various ethnic fermented foods	<i>L. Brevis</i> PLA2, <i>L. paracasei</i> PLA8, and <i>L. Brevis</i> PLA16	North-western Himalayas	[91]
Dosa batter	<i>Enterococcus hirae</i> LD3	Rice/South India	[110]

Name of Fermented Product	Potential probiotic-isolated LAB strains	Major cereal as substrate/ Sample Collection area	Reference
Sorghum-based traditional foods	<i>Last. Plantarum</i> , <i>Last. pentosus</i>	Sorghum/ Karnataka	[111]
Uttapam batter	<i>Lactobacillus pentosus</i> SJ65	Rice/ Assam	[112]
Dosa batter	<i>Lactobacillus Plantarum</i> LD1	Rice/South India	[87]
Marcha	<i>Lactobacillus plantarum</i> DM5	Wheat/Sikkim	[94]

Recently, Ahire *et al.* (2021) reported that *Last. plantarum* UBLP40 strain isolated from traditionally fermented idli batter showed good probiotic properties *in vitro* [9]. This strain showed 73% survival at pH 2.0 for up to 3 h, high survival in synthetic gastric juice (83%), and good survival in the media having pancreatin bile and an alkaline environment of intestinal fluid. *Last. plantarum* UBLP40 showed cell surface properties, inhibited the growth of pathogens, and had good *in vitro* antioxidative effects. The strain showed 48.59 U/mg phytase activity and medium bile salt hydrolase (BSH) activity. The isolated strain was safe *in vitro* antibiotic susceptibility profiling and non-hemolytic activity. Nath *et al.* (2021) isolated the efficient potential probiotic strain *Weissella confusa* strain GCC_19R1 from traditionally fermented sour rice, and the Cachar district of Assam, India, was chosen for sample collection [75]. In another study, *Pediococcus pentosaceus* OBK05 strain isolated from fermented products was evaluated for probiotic attributes, including cholesterol reduction ability *in vitro* studies [113]. Thakkar *et al.* (2020) demonstrated that *Lactobacillus fermentum* PD2 and PH5 strains isolated from traditionally fermented food products were efficient in decreasing serum cholesterol, low-density lipoprotein-cholesterol (LDL), and triglycerides in mice model in comparison to those fed the same high-cholesterol diet but without LAB supplementation [29]. *Lactobacillus fermentum* MJM60397 was reported to significantly reduce the total cholesterol and LDL-cholesterol concentrations in mice livers, which was attributed to its bile salt deconjugating activity [114]. Damodharan *et al.* (2016) demonstrated that *Lactobacillus helveticus* strains KII13 isolated from fermented products reduced the cholesterol in the animal model (mice) study. They demonstrated that the strain has the potential to produce antihypertensive peptides and reduce serum cholesterol [115].

Mishra *et al.* (2018) reported isolating *Lactobacillus* species from naturally fermented ethnic foods of the Garo Hills region of Meghalaya and characterized the selected isolates to explore their probiotic potentials [116]. Similarly, Kumari *et al.* (2016) conducted a study in which 51 isolates were isolated from traditionally fermented foods and beverages of the North-western Himalayas and evaluated for various probiotic attributes [91]. Three isolates (*L. Brevis* PLA2, *L. paracasei* PLA8, and *L. Brevis* PLA16) were the most technologically relevant and promising probiotic candidates compared to commercial probiotic strains. These studies confirmed the potential use of these strains for developing novel value-added functional foods as probiotics for the human health benefits of India's northeastern states. However, no further studies were reported to validate its probiotic properties *in vivo* after using it in product manufacturing.

A traditionally fermented wheat-flour dough known as Babroo from different locations of the Himalayan province (Himachal Pradesh) was used to isolate probiotic strain [93]. In this study, seven potential isolates were tested for various probiotic parameters. The results were assessed using principal component analysis (PCA), which showed that *Lactobacillus*

plantarum K90, *L. fermentum* K75, and *L. fermentum* K78 can be used as potential probiotic candidates. However, there were no safety studies of these cultures, and clinical trials need to be done to confirm their potential probiotic beneficial health effects. Yadav *et al.* (2016) demonstrated the probiotic potential of *Lactobacillus* (*L. plantarum* RYPR1) isolated from an indigenous fermented beverage called Raabadi (pearl millet-based) from five districts in Haryana [100]. Gupta and Tiwary (2015) reported isolating a strain *Enterococcus hirae* LD3 from dosa batter, which showed good probiotic potential in different *in vivo* probiotic tests [110]. Gupta and Tiwari (2014) demonstrated the probiotic properties of the isolated strain *Lactobacillus plantarum* LD1, isolated from dosa batter, a south Indian staple food [87]. Uttapam batter, a South Indian food reported to contain the bacteriocin-producing strain *Lactobacillus pentosus* SJ65 [112].

Rao *et al.* (2015) reported biochemical, physiological, and genetic characterization of LAB isolated from Sorghum-based traditional foods, and samples were taken from households in the Gulbarga region of Karnataka. The isolated LAB (*Last. plantarum* and *Last. pentosus*) strains showed good probiotic properties under *in vitro* conditions; however, more research is needed to establish the strains' beneficial effects on human health [111]. Das and Goyal (2014) isolated a novel strain *Lactobacillus plantarum* DM5 from the fermented beverage “Marcha” of Sikkim and evaluated its probiotic attributes by *in vitro* tests [94]. The strain showed a sufficient level of survivability in simulated gastrointestinal tract conditions and low pH, sufficient bile tolerance (0.5%), and lysozyme activity (200 mg/ml). The strain showed good cell surface hydrophobicity (53%), autoaggregation (54%), and bacteriocin activity. These qualities make isolate *L. plantarum* DM5 an excellent candidate to use as probiotics in novel functional foods or nutraceuticals. However, the strain may be used to develop a probiotic strain for industrial applications after clinical trials and proper validation.

6. Significance of LAB in Spontaneously Fermented Cereal-based Foods

6.1. LAB to extend shelf-life and improve organoleptic properties.

Fermentation of cereals has been used to get various value additions in raw ingredients like improvements in nutritional value, sensory and textural properties, shelf life, safety, digestibility, and consumer acceptance of food [7]. LAB, used to produce new and traditional fermented products having enhanced nutritional and traditional attributes, often produces antimicrobial compounds and antifungal agents [1]. Some LAB and yeast have antifungal and antimycotoxin-producing abilities, which are very important properties to control or minimize the public health hazard of mycotoxin [117,118]. Consumers primarily decide the acceptability of food products based on flavor, aroma, and overall acceptability [119,120]. The fermenting microbes, including LAB, form various volatile and non-volatile aroma-producing compounds, which is considered a safe way of producing organoleptically superior food products [121,122]. This property is considered one of the important selection criteria for any LAB strain to be an ideal probiotic for further use in cereal fermentation [47,74,123].

6.2. LAB to improve nutritional value and compositional changes.

Fermentation in cereals improves nutritional value and compositional changes through a series of biochemical pathways carried out primarily by microbial enzymes associated with

LAB. The protein and carbohydrate digestibility was reported to be enhanced by LAB [1,124]. The carbohydrates in food are very important sources of energy, and their proper digestion is necessary for the smooth functioning of the body. LAB converts starch into lactic acid in the fermentation process and helps to interact between the starch and gluten of cereal. This phenomenon leads to a lowering the Glycemic index and starch availability of the food product [80]. The gene *amyA* is found in a few LAB species of the genus *Lactobacillus*, *Lactococcus*, and *Streptococcus*, which causes the conversion of starch into lactic acid (known as amyolytic activity). Struyf *et al.*, (2017) reported the development of wheat bread with 90% reduced sugar by using yeast culture (*Kluyveromyces marxianus* and *Saccharomyces cerevisiae*), whereas Moro *et al.*, (2018) studied the effect of yeast and LAB cultures in combination to develop dietic bread [125,126].

LAB has the ability to ferment dietary fibers and plant sugars that are not easily degraded by human enzymes in the large [1]. Human beings cannot synthesize sufficient levels of the vitamin, but it has a vital role in proper metabolic functioning. The ability of vitamin production by yeast or LAB is strain specific. So, LAB can be added as a starter or adjunct culture to enhance food's nutritional and quality attributes. Very few studies have been reported in this regard. Chandrasekar Rajendran *et al.* (2017) reported that preparation of idli batter (cereal legume-based fermented product) by using *Lactococcus lactis* N8 and *Saccharomyces boulardii* SAA 655 enhanced the quantity of vitamin-B2 and folate in the range of 40 to 90% [127]. Bhusan *et al.* (2021) evaluated the riboflavin-producing attributes of 4 *Lactobacillus* strains viz. *Lactobacillus plantarum* (BBC33, BBC32A, BIF34 and BBC32B) [128]. Guru and Viswanathan (2013) have reported riboflavin-producing probiotic *L. acidophilus* obtained from curd and cheese samples [129]. Verni *et al.* (2019) reported using *Cordyceps sinensis* for vitamin E enhancement in stale rice [130]. There was a 100% increase in vitamin E compared to the untreated control product (stale rice).

LAB is associated with the improvement and enhancement of phenolic contents of cereal products during fermentation [130,131]. The increase in phenolics by LAB depends on several factors, such as the fermentation environment (incubation time and temperature, acidity of finished product), type of LAB strain used, type of cereal, and grain composition [1,132–134].

Some strains have been reported to produce γ -aminobutyric acid (GABA), which acts as a bioactive component in cultured cereal-based foods to improve cardiovascular functionality [21,135]. A few studies also demonstrated the production of bioactive fatty acids like conjugated linoleic acid and conjugated linolenic acid during the fermentation of cereals by LAB [136,137]

6.3. LAB to reduce antinutrients - phytates and mycotoxin.

Phytate (as myo-inositol 1,2,3,4,5,6-hexaphosphate), polyphenols, and tannins are antinutrients found in cereals and legumes [6]. There are various studies that reported that phytase produced by microbes converts phytate to myo-inositol and inorganic phosphate. Sandez Penidez *et al.*, (2020) reported *Lact. plantarum* CRL1964 has the phytase activity of 10.13 U/mg [138], and Ahire *et al.* (2021) reported that *Lact. plantarum* UBLP40 showed 48.59 U/mg phytase activity [9]. This suggests that UBLP 40 can degrade phytate to myo-inositol and inorganic phosphate, thus improving dietary mineral bioavailability. The phytic acid level

decreased in Kinema by *Bacillus subtilis*, whereas its reduction was also reported in rabadi and idli [139].

Certain mold species of genus *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* are reported in cereals, which can form hazardous toxins (known as mycotoxin) and even show survival in various processing treatments [140,141]. The antifungal effects of different LAB are widely studied in different foods, particularly in dairy and bakery products. Some studies in the Indian context have been reported, like Varsha *et al.* (2015) extracted a molecule, namely 2, 4-di-tert-butyl phenol (2,4 DTBP), and tested for its antifungal activity [142]. Gupta & Srivastava *et al.* (2014) reported that an antimicrobial molecule (AMPs LR14) had been purified and tested for antifungal activity against some of the predominant food spoilage molds (*Aspergillus niger*, *Rhizopus stolonifer*, *Mucor racemosus*, and *Penicillium*) obtained from *Lactobacillus plantarum* strain LR/14 [143]. Ahmad Rather *et al.* (2013) isolated the different LAB from the kimchi and subsequently tested these isolates against *Aspergillus niger* for primary screening. *Lactobacillus plantarum* YML007 showed the highest antifungal activity among tested isolates [144]. Further, this efficient strain showed antifungal activity (against *Aspergillus oryzae*, *Fusarium oxysporum*, *Aspergillus flavus*) and antimicrobial activity against other food-borne pathogens. The inhibitory activities were evaluated using scanning electron microscopy (SEM).

6.4. LAB as a producer of antimicrobial agents (Bacteriocin).

Bacteriocins are synthesized in the ribosome of the cell and are inhibitory to various types of gram-positive bacteria [145–149]. Most bacterial and archaea cells have the ability to produce at least one bacteriocin [150]. However, most of the studies for the production and application of bacteriocin are associated with Gram +ve bacteria, specially LAB [151]. Kumar *et al.* (2018) reported a bacteriocin-producing strain *Pediococcus pentosaceus* which showed broad-spectrum antimicrobial activity against foodborne pathogens isolated from “Appam batter” a south Indian rice-based traditionally fermented food [109]. Srinivasan *et al.* (2013) also demonstrated the antimicrobial effect of bacteriocin produced by isolated *Lactobacillus rhamnosus* L34 against various gram-positive and gram-negative pathogens as well as against food spoilage microbes. However, the isolated strains further need to be subjected to *in vivo* trails before use at a commercial scale [152].

6.5. LAB as exopolysaccharide producer.

Some LAB species produce exopolysaccharides during fermentation which helps to improve the textural and rheological properties of fermented foods without concern [153]. The product prepared using EPS-producing strains was reported to show improved rheology, mouth feel, consistency, creaminess, and predictable firmness stability of fermented foods. It can even be used to make food packaging material [122,153,154]. Varied structures of EPS perform different roles in EPS-producing cells, which are still unknown and mostly strain-dependent [155]. These polymers provide effective protection of cells from adverse environmental conditions [156], are involved in the development of biofilm and cell recognition in natural habitats [157], and protection from toxic substances [22]. Recently, EPS production by LAB was reported to exert a beneficial effect on gastrointestinal bacteria and immunity [158] and was found to be associated with anticancer activity [22]. Probiotic survival may be dependent

on the number of EPS produced by the probiotic strain [159]. These various protective effects made EPS production an important probiotic attribute for commercial probiotic strains [160]. LAB genera such as *Lactobacillus*, *Leuconostoc*, *Streptococcus*, *Weissella*, *Lactococcus* are known to produce EPS, and *Weissella* genus is widely studied for application in foods to enhance functionality [161]. Recently, idli batter, an acidic naturally fermented product, was used to isolate exopolysaccharides producing strain *Leuconostoc lactis* KC117496 [162], which was further tested for antioxidant properties [163]. Earlier, *Lactobacillus plantarum* 86 and *Weissella cibaria* 92 was also reported from idli and dosa batter [164]. Joshi & Kojiam (2014) reported two EPS-producing strains *Leuconostoc mesenteroides* (Yield - 904 mg/l), *L. lactis* (Yield - 340.82 mg/l) from naturally fermented alcoholic cereal-based beverage Kyiad pyrsi [165]. However, there are several reports about the isolation and use of EPS-producing strains in different food matrices, but the extensive investigation is still needed for certain aspects to ensure food safety before the commercialization of food [160].

7. Application of Starter Culture in Cereal-Based Fermented Foods

The interest of researchers and the fermented product industry is growing as LAB also exerts beneficial effects by enhancing good intestinal tract bacteria. Fermented dairy products are traditionally marketed as probiotic carriers due to their favorable nutritional content and sufficient consumer demand. However, increasing health awareness and rapid urbanization demands non-dairy fermented products, as dairy products have negative health effects due to lactose and cholesterol content [73]. The cereal-based probiotic products are considered excellent alternatives due to their nutritional and prebiotic content, which favors the growth of good bacteria and overcomes the adverse health effect of milk-based products.

Fermented cereal-based products are traditionally produced by spontaneous fermentation or back slopping and contribute to the safety and diversity of dietary intake of the human being. However, this uncontrolled fermentation leads to inconsistent quality, risk of batch failure, and food safety concerns [119]. Carefully selected autochthonous strains with desirable typical attributes with predictable quality characteristics based on scientific evidence are good alternatives to ferment cereals. Several factors are considered for the selection of multifunctional strain for manufacturing of these products, like safety [166], fermentation ability [167], organoleptic properties [122,161,168], antagonistic activity [169,170], nutritional properties [134,171,172], and production of bioactive substances [24,173].

8. Experimental Cereal-Based Probiotic Foods

Indian fermented products have various health benefits with potential probiotic strains [23,89,90,174]. The development of novel cereal-based probiotic foods depends on the desire for healthy and naturally preserved food, lowering the cost of health care and quality of life [32]. Some studies have been done for Indian cereal-based fermented products as the probiotic vehicle. Recently, Pintua & Verma (2019) reported optimizing sorghum-based fermented drinks, which had a shelf-life of seven days under refrigeration conditions (5–7°C) [175]. The developed Rabadi-like drink had maximum acceptability with 4.7% flour and 69% water based on curd. Other researchers also tried different products like wheat-based fermented beverages [176]; pearl millet-based fermented raabadi [177,178]. Rathore *et al.* (2012) used three kinds of cereal (malt, barley, and mixed barley-malt) for fermentation with probiotic strains

Lactobacillus plantarum (NCIMB 8826) and *Lactobacillus acidophilus* (NCIMB 8821) to study the effect of the media/substrate, functionality, and growth viability [179]. The study suggested that cereal-based media is a suitable substrate for growth, functionality, and sensory attributes for developing probiotic drinks. The various reports suggested that probiotic count should be not less than 10^6 cfu/g, or daily intake dose should be about 10^8 cfu/g [40]. A high concentration of probiotics is needed to compensate for the cell losses that occur during passage through the gastrointestinal tract.

9. Commercial Potential of Cereal-based Fermented Foods

There is a global demand for functional foods, and fermentation is a low-cost, environmentally friendly biotechnological method. The demand for functional foods is increasing at 8.6% annually worldwide in terms of market value [180], which compelled commercial manufacturers to develop novel products to fulfill consumers' expectations to survive in the most competitive market arena. Europe and Asia have long and huge traditions for producing LAB-fermented foods, and improving LAB starter cultures can help to improve the hygiene, safety, and quality of fermented foods [50].

The global probiotics market was valued at USD 58.17 billion in 2021, with a compound annual growth rate (CAGR) of 7.5% predicted from 2021 to 2030 (Grandview Research, 2021) (Grandview Research, 2021) (Grandview Research, 2021) (<https://www.grandviewresearch.com/industry-analysis/probiotics-market>, assessed on January 25, 2022). The market is divided into three categories: probiotic food and drinks, probiotic dietary supplements, and probiotics for animal feed. Dairy, non-dairy, cereals, baked goods, fermented meat, and dry food probiotics make up the food and beverage segment. With a more than 75% revenue share in 2021, the probiotic food and beverage category topped the global market (GrandViewResearch). Furthermore, the probiotic products can be bifurcated on the basis of ingredient microbe viz. bacteria (*Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and others) and yeast (*Saccharomyces boulardii* and others). A major part of probiotics are bacteria and contribute to about 80.0% share of this segment [182]. The human probiotic contributes more than 90% of revenue share globally on the consumption basis segment. The Asia-Pacific region accounts for more than 40% of the global probiotics industry's revenue [183]. Demand from countries like India, China, and Pakistan helps to drive overall growth. Rapid advances in science and technology, rising healthcare expenses, changes in food regulations affecting label and product claims, an aging population, and a growing interest in achieving wellness via diet are just a few drivers driving regional market expansion. There is a significant increase in consumer acceptance and knowledge due to the competitive marketing strategies of global probiotic industries.

The quality of commercial probiotics is still a problem in many nations worldwide, including Europe, the United States, South Africa, Taiwan, Pakistan, and India. According to research from these countries, the contents do not match the label information regarding identity, viability, number of microbial cells, activity, and purity [183]. Due to a lack of technology in the past, the market was swamped with probiotics that were unstable at room temperature. This problem was resolved as major global players devised technology, such as microencapsulation, which improves the stability and longevity of probiotic strains during storage. Furthermore, continuous research and development activities for the development of

efficient strains with a broad spectrum of treatments and the ability to endure high temperatures are projected to aid regional market expansion throughout the projection period.

10. Challenges and Future Perspective of Cereal-based Fermented Foods

One of the major challenges in developing cereal-based probiotic foods for Indian people is ignorance of these foods' health implications and nutritional value. The lack of knowledge of probiotics is also a concern that makes people skeptical about probiotic-fermented products. Most of the research for probiotics has been done on milk-based products, and very few have been tried in plant-based food matrices in India. Cereals and pseudo-cereals grains are complex substrates, and the growth pattern of probiotics is different in this media as compared to milk substrate. Furthermore, the lack of systematic research to characterize probiotic growth patterns and survivability in cereal substrates is still challenging for the Indian scientific community. Another important concern is the lack of production facilities in controlled conditions and the availability of pure starter culture at an affordable price to the mass population. In India, most cereals-based fermented products are produced by spontaneous fermentation or back slopping, leading to variation in product quality and functional characteristics in each batch. Therefore, sufficient pure starter cultures are needed for desired fermentation to obtain quality products in terms of sensory, nutritional value, and functionality.

Probiotics are gaining attention from researchers worldwide due to their beneficial health implications. Since the dawn of civilization, probiotics have been consumed in fermented products, but scientific research has recently begun. Sufficient research suggests using probiotic LAB to cure GIT abnormalities and preserve pathogenic colonization in the gut. It is also used as a natural antimicrobial agent (bacteriocins as biopreservative) as an alternative to antibiotics and chemical preservatives. Therefore, rural and urban people should be encouraged to continue using traditionally fermented products to obtain the beneficial effect of these products until the availability of commercial probiotic products at affordable prices. The cereals also contain various probiotic ingredients, which help stimulate the growth of probiotics. This combination will help higher survival and growth of probiotics in the food matrix and gastrointestinal tract. Most Indian cereal-based fermented foods are good sources of prebiotics and probiotics to exert beneficial effects on the host.

Recent advances in molecular and metagenomics techniques pave the prospect of isolating, identifying, and characterizing potential probiotic LAB strains from traditionally fermented cereal-based products of the Indian subcontinent. These strains can be harnessed to develop novel probiotic products for the benefit of the human being. The uses of indigenous probiotics in cereals are an emerging area of research to develop novel functional products to cure gastrointestinal disorders and other health benefits. Furthermore, the indigenous strains obtained from locally fermented products with proven probiotic attributes can be made available to rural masses for low-cost production, controlled fermentation, and a sense of safety for the product.

Another prospect of probiotics is continuous research to make efficient strains and comply with scientific and regulatory definitions. The LAB strains can exist and survive sufficiently in fermented cereal products until the expiry of products in traditional Indian foods. The strains should be explored for the production of other beneficial metabolites in comparison to dairy-based products. The metagenomics approach can play an important role by providing

insight into the genes, structure, and function of cereal-based fermented products prepared using probiotic microbes. Another important aspect is to assess the performance and suitability of each probiotic strain isolated from India's spontaneously fermented cereal products. This will provide the best insight into selecting the most appropriate probiotic starter culture strain for product preparation.

11. Conclusions

Probiotic bacteria are most commonly isolated from the gastrointestinal tract of humans. The significance of the emergence of health-promoting strains is underappreciated. Although most probiotic meals are now dairy-based, there is rising interest in isolating probiotic strains from various dietary sources (fruits, vegetables, legumes, and cereal foods) that may be potential carriers of probiotics, prebiotics, and bioactive substances. At the same time, identifying and characterizing probiotic strains from various habitats may significantly enhance the value of such bacteria in the future. Future research and advancements in functional cereal-based foods may involve diversification other than dairy products. They are a good alternative to dairy matrices because the fermentation of cereal leads to improved nutritional and functional quality, enhanced shelf life, safety, and digestibility due to the formation of organic acids and other bioactive agents. Moreover, all the cereal-based fermented products of India reviewed have LABs with probiotic potential, which can be used to produce novel food and pharmaceutical products. However, to substantiate the beneficial effects of cereal-based fermented products and isolated potential probiotic strains, further *in vivo* trials need to be evaluated, including fulfilling the criteria of human clinical trials in different cereal matrices and inoculum doses in different concentrations.

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Conflict of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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