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A review on millet based probiotic beverages

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Abstract
Probiotics are the live microorganisms which beneficially affect the host by improving its intestinal microbial balance. Probiotic products available in market are generally milk and yoghurt based, but nowadays due to increase in peoples veganism or vegetarianism throughout the world, demand for non-dairy probiotic beverage and also the people's health problems like lactose intolerance and blood cholesterol related diseases it is need to develop non-dairy probiotic beverages by using different substrates other than milk. So for this reason there is need to search the alternative raw material for probiotic microorganisms. Considering the nutritional value and other facts about cereals and millets, these can be useful raw material for probiotics. This review aims at highlighting the research done on non-dairy probiotic beverage particularly from cereals and millets and also the health benefits of the probiotics on human health.

Keywords: Probiotic, millets, non-dairy probiotic beverage

Introduction
The most probiotic food at the market are milk based and very few are made for development of probiotic food using other substrate such as cereals. Their large distribution and important nutritive value are focused the attention on their use as raw materials for development of new fermented functional food products (Angelov *et al*., 2006) [2]. The term "probiotics" was first introduced in 1965 by Lilly and Stillwell; in contrast to antibiotics, probiotics were defined as microbial derived factors that stimulate the growth of other organisms. Probiotics are live microbes that can be formulated into many different types of product, including foods, drugs, and dietary supplements. Regular consumption of high levels of probiotic bacteria is required to confer health benefits. It is important that the organisms remain viable in the food product until the time of consumption and be present in significant numbers in order to confer benefits to the consumer.

Probiotics have been defined as living bacteria and supportive substances that have beneficial effects on the host by improving the bacterial balance in the intestine (Fuller, 1991). This definition was later expanded to include living bacteria or mixed bacteria that have beneficial effects on the gastrointestinal and respiratory system of the host by improvement of the balance of intestinal flora (Salminen *et al*., 1998) [25]. Recently, probiotics have been more widely defined as bacteria that work to maintain the host's health. There are some ideal properties of the probiotic strains, which would benefit the human health and could be used in probiotic industry: resistance to acid and bile; attachment to the human epithelial cells; colonization in the human intestine; production of antimicrobial substances, called bacteriocins (Jack *et al*., 1995) [16].

Cereal grains (Millets) had been one of man's earliest sources of food. One way of processing the grains into food is through fermentation or by inoculation of the probiotic microorganisms. Fermented foods contribute to about one –third of the diet worldwide (Campbell 1994) [4]. Millet is general category for several species of smell grained cereal crops and is a food staple in parts of India, Africa, China and elsewhere. Millets have been cultivated since prehistoric times in regions of North Asia, though its origin ambiguous.

Millets and millet based beverages have been showed as appropriate for probiotic products. Also they have several functional food components such as minerals, vitamins, dietary fiber. In recent years, studies on millet based probiotic beverages such as finger millet, pearl millet have been performed in conjunction with different probiotic strains and obtained appealing results (Farseen *et al*., 2017) [9].

Cereals and Millets Based Probiotic Beverages

Cereals and Millets are also a substrate that has been used for the production of probiotic products because cereals, millets and legumes have nutritional content like fibers that act as prebiotics and act as food for the probiotics and also protect probiotic cells from the adverse conditions of the gastrointestinal tract. Nutritionally they provide proteins, carbohydrates, vitamin B and E, Iron, trace minerals and most important fiber. In addition consumption of cereal has been associated with the risk reduction of several chronic diseases.

Boza

It is popular fermented beverage consumed in Kazakhstan, Kosovo, Azerbaijan, Bulgaria, Albania, Turkey and Romania. It is a malt drink made by fermenting various grains. Different grains are used in different countries. Microflora identification of Bulgarian boza shows that it mainly consists of yeasts and lactic acid bacteria, in an average LAB/yeast ratio of 2.4. The lactic acid bacteria isolated were *Lactobacillus plantarum*, Lb. acidophilus, *Lactobacillus fermentum*, *Lactobacillus coprophilus*, *Leuconostoc reffinolactis*, *Leuconostoc mesenteroides* and *Lactobacillus brevis*. The yeasts isolated were Saccharomyces cerevisiae, *Candida tropicalis*, *Candida glabrata*, *Geotrichum penicillatum* and *Geotrichum candidum* (Anglov *et al*., 2000; Blandino *et al*., 2003) [3].

Pito

This drink is mostly consumed by Benin people in the mid-western and middle Belt region of Nigeria. It is prepared by using cereal grains like maize, sorghum, or blend of both. The malted grains are mixed with water and then boiled, then it is fermented overnight and this become sour due to microbial action. (Ukwuru *et al*., 2018) 27[].

Bushera

It is a cereal based traditional beverage prepared in the Uganda in the districts of Kabale and Rukungiri. Thesorghum, or millet flour from the germinated sorghum and millet grains is mixed with the boiling water and left to cool to ambient temperature. Germinated millet or sorghum flour is then added and the mixture is left to ferment at ambient temperature for 1 –6 days. The lactic acid bacteria isolated from Bushera comprised of five genera, Lactobacillus, Lactococcus, Leuconostoc, Enterococcus and Streptococcus. Lb. brevis was more frequently isolated than other species (Muyanja *et al*., 2003) [20].

Mangisi

It is sweet sour beverage prepared by natural fermentation of millet mash (Zvauya *et al*.,1997) Finger millet is malted then milled and flour mixed with water. Then the mixture is slowly heated for 80°C to almost boiling. This beverage comprised of LAB species.

Mahewu

It is a lactic fermented nonalcoholic beverage made from the maize porridge, which is mixed with the water. The sorghum, millet malt, or wheat flour is then added and left to ferment. It is consumed in Africa and some Arabian Gulf countries. The spontaneous fermentation process is carried out by the natural flora of the malt at the ambient temperature. The predominant microorganism found in African mahewu is *Lactococcus lactis* sub sp. Lactis (Blandino *et al*., 2003) [3].

Pozol

This is the beverage, consumed in the Southeastern Mexico, is made by cooking maize in lime solution, washing with water, grinding to make a dough known as nixtamal, shaping into balls, wrapping in banana leaves and leaving to ferment at ambienttemperature for 0.5–4 days. The fermented dough is suspendedinthe water and drunk. Some fibrous components are not completely solubilized by nixtamalization and sediment is present in the beverage when the dough is suspended in the water (Wacher *et al*., 2000) [29].

Obiolo

It is fermented beverage made from millets and sorghum malts in Nigeria. *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactococcus lactic* and *Bifidobacterium longum* was isolated from this beverage.

Togwa

It is very similar to mahewu. It is a traditional beverage consumed in Africa, is usually made from the maize flour and finger millet malt. In this region, it is consumed by the working people and also used as refreshment as well as a weaning food (Oi and Kitabatake, 2003). Togwa is also regularly consumed by the young children. The cereal or cassava flour is cooked in the water. After cooling at 35 ^{0}C, starter culture (old togwa) and cereal flour from the germinated grains are added. The fermentation process finishes at pH 4.0–3.2 (Molin, 2001) [19]. Lactic acid bacteria can rarely convert starch intolactic acid, however, some strains of Lactobacillus and Streptococcus (Parada *et al*., 1996), for example, Lb. plantarumA6, isolated by (Giraud *et al*., 1993) [13] showed extracellular amylase activity. Sour cassava starch is obtained by a natural fermentation and this product is largely appreciated in Africa, South America and other developing countries.

Burukutu

This is the popular alcoholic beverage of vinegar like flavor, sweet, sour taste mostly consumed in Nigeria and Ghana. In the preparation of burukutu sorghum is used by soaking it overnight and then spread in tray covered with banana leaves and allow them to germinate. After germination the malt is prepared. This malt is mixed with cold water and boiled for 6-12 hrs after which is filtered through fine mesh. The filtrate is fermented overnight. It is then boiled again to concentrate it and then cooled. (Ukwuru and Ohaegbu, 2018) [27] *Lactobacillus* spp., *Leuconostoc mesenteroides* and *acenetobactor* are produced after fermentation. (Ukwuru *et al*., 2018) [27].

Santos

Developed a probiotic beverage with the fermented cassava flour using mixedculture of *Lb. plantarum*, which were amylolytic strains of Lb. casei Shirota and Lb. acidophilus. The best parameters of the fermentation were 8% inoculation rate, incubation temperature and period as 35 ^{0}C and 16 h, respectively, and 20% of cassava flour. The lactic beverage maintained its microbiological and physico-chemical quality for 28 days storage period at 4 ^{0}C.

Other probiotic Beverages

The beverage produced by the use of Finger millet based probiotic beverage *using Lactobacillus casei* (Farseen *et al*., 2017) [9].

(Angelov *et al*. 2006) [2] produced a symbiotic functional drink from the oats by combining a probiotic starter culture and whole-grain oat substrate.

Microorganisms commonly used as probiotics (Ivonne F. *et al*., 2011)

Lactobacilii:

Lactobacillus plantarum
Lactobacillus fermentum
Lactobacillus plantarum
Lactobacillus brevis
Lactobacillus acidophilus
Lactobacillus rhamnosus
Lactobacillus gasseri
Lactobacillus bulgaricus
Lactobacillus crispatus

Bifidobacterium:

Bifidobacterium bifidum
Bifidobacterium longum
Bifidobacterium infantis
Bifidobacterium lactis
Bifidobacterium breve
Bifidobacterium animalis
Bifidobacterium adolescentis

Others

Saccharomyces boulardi
Lactococcus lactis
Propino bacteria

Probiotic Lactic Acid Bacteria in Millets

The experiments conducted on Lactic acid bacteria that they are Gram positive, non-spore forming catalase negative cocci or rods that are anaerobic, micro-aerophilic or aero-tolerant. These microorganisms produce lactic acid as the sole, major or an important product from the energy yielding fermentation of sugars. They include the genus *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Bifidobacterium*, some Enterococcus and some streptococcus. Some yeast such as saccharomyces and moulds such as penicillium, aspergillus and botrytis too produce lactic acid. It is found that the lactic acid fermentations provide foods that have a variety of flavors, aromas, textures in addition to the foods being safe and having a long shelf-life. However, not all lactic acid bacteria are useful; some are involved in food spoilage due to action of proteinases and lipases that degrade proteins and lipids, respectively, producing by-products that cause off-flavours. *Pediococcus damnosus* has been reported to produce off-flavour in beer while *L. bifermentans* and *L. alimentarius* are associated with spoilage of refrigerated and packaged foods. Some are pathogenic especially species belonging to the genera Streptococcus (cause throat infections) and Enterococcus. (Wood and Holzapfel, 1995) [30].

Studies conducted on the growth of potentially probiotic Lactic acid bacteria in cereal based substrates. In this study, the growth characteristics of four potentially probiotic lactic acid bacteria in cereal substrates were investigated. A general conclusion is that in a non pH controlled lactic acid fermentation of cereals the main inhibitor of microbial growth are pH and nutrient limitations, probably sugars (Charalampopoulos *et al*., 2001)

Health Benefits of Probiotic Food

Consumption of probiotics is related with a range of health benefits including stimulation of the immune system, protection against diarrheal that is digestion system diseases and nosocomial and respiratory tract diseases, decrease in cholesterol, and anticancer properties.

It is stated that the Lactic acid fermentation improves usually the nutritional value and digestibility of cereals. Cereals are limited in essential amino acids such as threonine, lysine, and tryptophan, thus making their protein quality poorer compared with animals and milk. (Chavan *et al*., 1989) [7]. It is reported improved minerals availability of pearl millet fermented with pure cultures of lactobacilli and yeasts. (Khetarpaul *et al*., 1990) [17].

Vanderhoof (2000) [28] analyzed that Lactobacillus plantarum reduced incidence of diarrhoea in daycare centres when administered to only half of the children Especially effective in reducing inflammation in inflammatory bowel; e.g., enterocolitis in rats, small bowel bacterial overgrowth in children, pouchitis reduced pain and constipation of irritable bowel syndrome reduced bloating, flatulence, and pain in irritable bowel syndrome in controlled trial. Positive effect on immunity in HIV+ children. It is observed strain of Lactobacillus acidophilus significant decrease of diarrhea in patients receiving pelvic irradiation decreased polyps, adenomas and colon cancer in experimental animals Prevented urogenital infection with subsequent exposure to three pathogens *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, Lowered serum cholesterol levels (Parvez *et al*., 2006) [24]. Several probiotic strains, especially *Lactobacillus rhamnosus* have been shown to prevent or alleviate infantile diarrhea, caused mainly by rotavirus. It is also well-established that some probiotic strains can both prevent and shorten antibiotic-associated disorders. (Fuller *et al*., 2008) [12].

Most of the probiotics have been shown to produce antipathogenic compounds ranging from small molecules to bioactive antimicrobial peptides. The decrease of pH by acids like lactic and acetic has bactericidal and bacteriostatic effects (Agarwal., 2005). Probiotics can produce antimicrobial peptides and bacteriocins, the antimicrobial action of the compounds involves increased permeability of the cytoplasmic membrane of the target cells, which leads to the release of small cytoplasmic particles, depolarization of the membrane potential and eventually to cell death (SimovaED *et al*., 2009).

Many human studies performed to investigate the effects of probiotic cultures on the immune system reveal that probiotic bacteria are able toenhance both innate and acquired immunity by increasing natural killer cell activity and phagocytosis, changing cytokine profiles, and increasing levels of immunoglobulins. Two probiotic strains have been developed with a particular focus on their enhancing effects on immune responses: *Bifidobacterium lactis* and *Lactobacillus rhamnosus*. Both strains have been demonstrated in several studies to enhance natural immune function in healthy people (Fuller *et al*., 2008) [12].

The innate immune system via toll-like receptors recognizes a large group of chemical structures in pathogens and lipoteichoic acids which enables them to recognize foreign objects which trigger a cascade of immunological defense mechanisms (Vasilijevic. 2008). Probiotics can influence the immune system by products like metabolites, cell-wall components (peptidoglycanes, lipopolysaccharides) and DNA. Probiotic products are recognized by host cells

sensitive for these because they are equipped with recognition receptors (Vanderpool C *et al*., 2008).

There is growing evidence that probiotics have potential therapeutic benefits for patients suffering from IBD. Controlled clinical studies have demonstrated that probiotics are efficacious in the maintenance of remission of pouchitis, prophylaxis of pouchitis after the formation of an ileoanal reservoir, maintenance of remission of ulcerative colitis, and treatment of Crohn's disease (Fooks *et al*., 2002).

Molin (2001) [19] found that in humans, *L. plantarum* 299v can increase the concentration of carboxylic acids in feces and decrease abdominal bloating in patients with irritable bowel disease. It can also decrease fibrinogen concentrations in blood. Should probiotics be administrated through foods, the probiotic organism must remain vigorous in the food until consumption and the food must remain palatable, i.e the food carrier and the organism must suit each other immunologic defense.

Bacterial cultures, yogurt starter cultures as well as some probiotic cultures are known to improve the lactose digestion in lactose maldigestors. Subjects suffering from lactose intolerance have a very low concentration of the lactose-cleaving enzyme β-galactosidase, and bacteria in fermented or unfermented food products release their β-galactosidase in the small intestine, where it supports lactose digestion (Li *et al*., 2012).

The use of probiotics to reduce the risk of hypercholesteremia seems very attractive especially if consumed as a part of a normal daily nutrition (Vasilijevic., 2008). It is found that *Lactobacillus rhamnosus* GG confers an immune stimulatory effect in healthy adults. Probiotics have also been used successfully in the management of atopic eczema in infants. Furthermore, *Lactobacillus rhamnosus* GG proved to be effective in the prevention of early atopic disease in children at high risk. The Lactobacillus rhamnosus GG product when given prenatally to mothers and postnatally for 6 months to the mothers or to their infants directly, reduced the frequency of atopic eczema in the probiotic group to half that of the placebo group at the age of 2 years. The preventive effect was reconfirmed at the age of 4 years (Delcenserie *et al*., 2010) [6].

A few epidemiological studies indicate that consumption of fermented dairy products containing *Lactobacilli* or Bifidobacteria, lowers the incidence of colorectal cancer. However, there is some indirect evidence based on several markers applied in human studies (e.g., fecal enzyme activities, fecal mutagenicity and genotoxicity, immunological markers), which shows that probiotics reduce the risk of colon cancer. A case-control study conducted in Japan with 180 cases and 445 controls revealed that habitual intake of lactic acid bacteria reduces therisk of bladder cancer (Rafter., 2004) [23].

Respiratory effects of probiotics in animal models have included attenuating allergic airway responses and protecting against respiratory pathogens. Dendritic cells appear central to directing the beneficial immune response to probiotic bacteria and in translating microbial signals from the innate to the adaptive immune system, whereas regulatory T cells are emerging as potentially key effectors of probiotic- mediated responses, particularly in the reduction of allergic inflammation. Despite progress in basic research, clinical trials of probiotics in allergy/asthma and respiratory infection have been highly variable at best, leading to an undermining of confidence in this potential therapeutic strategy (Forsythe., 2011) [10].

Studies carried out on the effects of lactic acid bacteria on constipation and intestinal motility, have shown reduced severity of constipation and an improved bowel movement frequency and stool consistency in constipated but otherwise healthy people after consumption of a fermented milk drink containing *Lactobacillus casei* strain Shirota. Administration of BifidobacteriumlongumBB536 to constipated women resulted in a significantly increased defecation frequency and stool softness. A positive influence of *Bifidobacterium longum* BB536 on the "regularity" was also reported for elderly people (Ouwehand *et al*., 2002) [22].

Studies suggest that probiotic preparations given orally or intravaginally may provide a therapeutic source of Lactobacilli to help control urogenital infections in women. A case-control study with 139 females with acute urinary tract infection and 185 control serve aled that consumption of fermented milk products containing probiotic bacteria was associated with a decreased risk of recurrence of urinary tract infection (Dani *et al*.,2002) [5].

Several probiotic strains have been shown to inhibit Helicobacter pylori *in vitro*, which has been associated with gastritis, stomach carcinoma, gastric ulcer, and lymphomas. Human studies confirmed this inhibitory effect on *Helicobacter pylori*, which seems to be independent of the viability of the bacteria (Hamilton-Miller *et al*., 2003) [14].

Conclusion

From above information it is concluded that, we can use millets and cereals as raw material for developing non-dairy probiotic beverage. Cereals and millet could be ideal substrates for the culture of probiotics, since they already contain beneficial nutrients like dietary fibers which acts as prebiotic that is food for the probiotics, antioxidants, minerals, vitamins etc. Also these are helpful for peoples having health problems like lactose intolerance and coronary heart diseases. Probiotic products available in market are generally milk based, however the people's demand for cholesterol free probiotic beverage encourages scientists and researchers to explore newer substrates for the probiotic microorganisms.

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