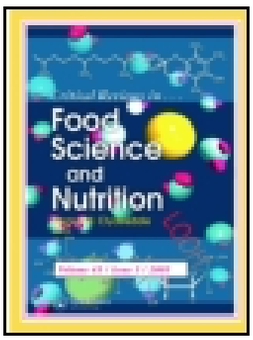

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Probiotic Potentials of Cereal-Based Beverages

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ABSTRACT

Probiotics offer remarkable potential for the prevention and management of various infective and non-infective disorders. They are reported to play key roles in the suppression of gastrointestinal infections, antimicrobial activity, improvement in lactose metabolism, reduction in serum cholesterol, immune system stimulation, antimutagenic properties, anti-carcinogenic properties, anti-diarrheal properties and improvement in inflammatory bowel disease. Although probiotic foods are classically confined to beverages and cheese, containing live organisms of the lactic acid bacteria family, such health-promoting foods are traditionally dairy-based, comprising milk and its fermented products. However, recent research focuses on the probiotic potentials of fermented cereal-based beverages which are especially consumed in developing countries characterized by low nutritional security and high incidence of gut pathogen infections. Moreover, lactose intolerance and cholesterol content associated with dairy products, coupled with the vegetarian tendencies of diverse populations in the third world, tend to enforce the recent recourse to non-dairy beverages. Probiotic microorganisms are mostly of human or animal origin; however, strains recognized as probiotics are also found in non-dairy fermented substrates. This review examines the potentials of some traditional cereal-based beverages to

serve as probiotic foods, their microbial and functional properties, as well as their process optimization and storage for enhanced utilization.

Keywords

Cereal-based beverages, fermentation, probiotic organisms, characterization.

1.0 INTRODUCTION

The Russian scientist, Elie Metchnikoff (1845-1916), is credited with introducing the concept that live microorganisms are beneficial for health. He suggested that by implanting lactic acid bacteria (such as Lactobacilli from a Bulgarian Yoghurt culture), the pathological effects of unwanted bacteria could be counteracted thereby having life expectancy prolonged. In 1965, this concept was formalized by introduction of the term "Probiotics" by Lilly and Stilwell to define growth promoting factors produced by microorganisms. Probiotics are defined as live microorganisms in foodstuffs which, when consumed at certain levels in nutrition stabilizes the gastrointestinal tract microflora thereby conferring health benefits on the consumer (FAO/WHO, 2002). It has been reported that populations of 10^6-10^7colony forming units (CFU)/g (or CFU/ml) in the final product are established as therapeutic quantities of probiotic cultures in processed foods (Talwalkar et al., 2004; Ukeyima et al., 2010), reaching 10^8-10^9CFU, provided by a daily consumption of 100 g or 100 mL of food. At the point of consumption, the level of probiotics in the food should be $\geq 10^6$CFU/ml (Table 1).

Bacterial strains most commonly used as probiotics belong to the genera *Lactobacillus* and *Bifidobacterium*, but other organisms are also applied such as *Lactococcus* and *Enterococcus* (Prado et al. 2008). A list of the major organisms commonly applied as probiotics is given in Table 1. Except for the genus *Bifidobacterium* which is part of the Actinobacteria phylum, these organisms belong to the lactic acid bacteria (LAB). Since bifidobacteria share many metabolic properties of LAB such as being Gram-positive, fermentative and producing lactate among other

acids, the term LAB is usually taken to also include bifidobacteria (Ukeyima et al., 2010; Vankerckhoven et al., 2008)

The intestinal tracts of newborns are colonized with *Bifidobacterium* within days after birth and the population is influenced by age, diet, antibiotics, and stress. The effectiveness of this organism is related to its ability to colonize the intestinal tract and control undesirable intestinal bacteria, but the adhesive factor and survivability are not presented in all isolated Bifidobacteria. Lactobacilli are typically characterized as Gram-positive, non-spore-forming, non-motile, catalase-negative bacteria growing under micro-aerobic or strictly anaerobic conditions. Some species produce lactic and acetic acids when they use glucose as a carbon source. One of the most important characteristics of a probiotic strain is that it must be non-pathogenic and GRAS (Generally Regarded As Safe). The probiotic must also present some desirable characteristics, such as low cost, maintain its viability during the processing and storage, facility of the application in the products and resistance to the physicochemical processing of the food (Prado et al. 2008). Strain affinity to particular food products is one factor that requires extensive post-laboratory studies.

Heller (2001) and Rivera-Espinoza and Gallardo-Navarro (2010) outlined some factors that may influence the survival and activity of a probiotic product when entering the consumer's gastrointestinal tract as including: (1) the physiologic state of the probiotic organisms added, given by the logarithmic or the stationary growth phase; (2) the concentration at the time of consumption, as several studies have revealed that some commercial products do not sustain adequate populations of viable probiotic bacteria during their shelf-life; (3) the physical conditions during product storage, since some probiotic microorganism have shown viability

during frozen shelf-life because they are held at lower temperature and subject to less temperature abuse; (4) the physico-chemical properties of the product to which the probiotics are added: pH, water activity; carbon, nitrogen, mineral and oxygen content affect the performance of these bacteria in many food environments and particularly in fermented foods; and (5) the possible interactions of the probiotics with the starter cultures (probiotics have been included as both starter and non-starter cultures in fermented dairy products), with regard to bacteriocin production, antagonism, and synergism. The interactions of probiotics with either the food matrix or the starter culture may be even more intensive when probiotics are used as a component of the starter culture (Heller, 2001). When cereal or cereal components are used as probiotic growth media, there are additional considerations along with those mentioned above. These include grain composition with associated processing requirement, probiotic strains that usually favor high-fiber substrates, organoleptic properties, and the nutritional value of the resulting product (Charalampopoulos et al., 2002b). For example, lactobacilli and bifidobacteria have complex nutritional requirements (carbohydrates, amino acids, peptides, fatty esters, salts, etc.) that vary widely from species to species. However, after testing several heterofermentative and homofermentative lactobacilli, researchers concluded that oats are, in general, suitable substrates for lactic acid bacteria (LAB) growth, regardless of differences between bacterial species (Lamsal and Faubion, 2009).

A question has always been raised: are probiotics regarded as functional ingredients in foods? Functional foods are commonly regarded as foods that promote human health over and above the provision of basic nutrition. A working definition, proposed by Stanton et al. (2005), describes functional foods as foods that can be satisfactorily demonstrated to beneficially affect one or

more target functions in the body, beyond adequate nutritional effects, so as to lead to an improved state of health and well-being and⁄or a reduction of risk of disease. The action of probiotics on intestinal microbiota results in vital benefits, including protection against pathogens, immune stimulation, prevention and treatment of gastrointestinal disorders, metabolism of lactose and positive effects on colonic health and host nutrition. Evidence for the role of probiotics in maintenance of health or prevention of disease is increasing and is supported, in some cases, by blind, placebo-controlled human trials (Sanders, 2003). There are some ideal properties of the probiotic strains, which would be of immense benefit and could be exploited in the probiotic food industry: resistance to acid and bile; attachment to the human epithelial cells; colonization in the human intestine; production of antimicrobial substances, called bacteriocins; and good growth characteristics. Some of the beneficial effects of probiotics on human health, which include increase of lactose tolerance and ingestion; positive influence on the intestinal microflora; reduction of intestinal pH; improvements in intestinal functioning; cholesterol reduction; reduction of ammonia and other toxic compounds; production of B-group vitamins (especially folic acid); restoration of normal intestinal microflora after antibiotic therapy; treatment and prevention of acute diarrhea caused by rotaviruses; and stimulation of the immune response (Prado et al., 2008; Gibson and Roberfroid, 1995) are viewed as both functional and nutritional, whereby the functional aspects are more pronounced. All these are evidences of the functionality of probiotic foods, and the use of probiotics as functional ingredients.

A number of fermented products based on milk or curd have been prepared by using probiotic micro-organisms (Sharp et al., 2008; Cruz et al., 2009), but until recently, much less work has

been done on the development of probiotic fermented products based on cereals which constitute the staple diet of the majority of populations in developing countries. Many of the bacteria used in probiotic preparations (*Bifidobacteria* and LAB) have been isolated from human fecal samples to maximize the likelihood of compatibility with the human gut microflora and improve their chances of survival. However, microorganisms isolated from fermented non-dairy foods, especially cereal and cereal-based products have shown these abilities in *in vitro* studies and using animal assays. Probiotics are associated with fermented foods and it is therefore of importance that spontaneously fermented foods, especially cereal-based beverages, that are so common in Africa and other parts of the developing world, be assessed for their probiotic attributes. In this paper, the use of cereals in fermented probiotic beverages is examined with emphasis on the involvement of known probiotic strains in the diverse fermentations, the optimization of process and storage conditions and the functionality of the products.

2.0 PROBIOSIS AND PROPHYLAXIS

Tolerance to the extreme gastrointestinal conditions (acid, bile, enzymes, low levels of oxygen), ability to adhere to the gastrointestinal mucosa and competitive exclusion of pathogens have been some criteria for probiotic selection (Collado, 2008) by *in vivo* or *in vitro* assays. The importance of the food carrier such as dairy products may enhance microbial survival in gastric juice, most likely due to a buffering or protective effect. Studies demonstrated the protective effect of Cheddar cheese, compared with yoghurt as a food carrier for delivery of viable probiotic lactobacilli and enterococci to the GIT (Ross et al. 2005). This effect may be due to the buffering capacity of the food product. Addition of milk or milk proteins to gastric juice or

media simulating gastric juice significantly increased the pH and enhances survival of some *Lactobacillus* and *Bifidobacterium* species.

Scientific evidence points to the fact that the ability of a probiotic bacteria to confer a health effect largely depends on the particular strain being used (Tuohy et al., 2007). There has thus been a resurgence of interest about the strain specific benefits of probiotics and clinical research is quickly accumulating to support the evidence for their use. Initial assessment of strains for use as probiotic cultures using such assays as acid and bile tolerance, can provide useful information for predicting their performance during gastric transit, and selection of strains based on tolerance to certain stresses, such as acid may also be useful predictors of technological performance in fermented foods. The ability of potentially probiotic strains to survive in acidic conditions has been investigated in a number of studies, which have shown great variation between strains and species (Ross et al., 2005). In some studies of strain-specific responses, no cells of *Lactobacillus acidophilus* culture were recovered following 45 min exposure to pH 2.0, while at pH 4.0 the number of cells was not significantly reduced after 2 h. Similar trends have been shown for survival of *Lactobacillus rhamnosus* GG in human gastric juice at pH values ranging from 1.0 to 7.0. In general, *Bifidobacterium* cultures are less acid tolerant than *Lactobacillus* cultures and this is reflected by their reduced tolerance to human gastric juice.

Probiosis has also been found to be enhanced by synbiosis. A synbiotic is defined as a mixture of a probiotic and a prebiotic that beneficially affects the host by improving the survival and the implantation of live microbial dietary supplements in the gastrointestinal tract and by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health promoting bacteria (Roberfroid, 1998). Such a combination aids survival of the administered

probiotic and facilitates its inoculation into the colon. The combination of probiotics and prebiotics as a single product benefits the gut bacteria by supplying its nutrients, which enables them to multiply rapidly in the gut and produce health benefits more effectively. According to Wang (2009), the effect of a prebiotic is, essentially, indirect because it selectively feeds one or a limited number of microorganisms thus causing a selective modification of the host's intestinal (especially colonic) microflora. It is not the prebiotic by itself but rather the changes induced in microflora composition that is responsible for its effects. Indeed, the most important bacterial genera targeted for selective stimulation are the indigenous bifidobacteria and lactobacilli.

The theoretical basis for selection of probiotic microorganisms includes safety, functional (survival, adherence, colonisation, antimicrobial production, immune stimulation, antigenotoxic activity and prevention of pathogens) and technological aspects (growth in milk, sensory properties, stability, phage resistance, viability in processes) (see Figure 1). The safety of probiotics in food and clinical use has been greatly underlined by carefully undertaken *in vivo* trials using volunteers. The functional aspects are confirmed by clinically validated and documented health benefits. Probiotics have also been shown to maintain viability during normal food processing, and are known to be stable during intestinal passage. To study the probiotic strain in the gastrointestinal tract, molecular techniques must be established for distinguishing the ingested probiotic strain from the potentially thousands of other bacterial strains that make up the gastrointestinal ecosystem (Saarela et al., 2000). Additionally, techniques are required to establish the effect of the probiotic strain on other members of the intestinal microbiota and importantly on the host. This includes not only positive health benefits, but also demonstration that probiotic strains do not have any deleterious effects.

2.1 Medicinal Values of Probiotics

There is some debate as to whether the concept of probiotic should be modified due to the prevalence of some situations in which health beneficial effects have been linked to non-viable cells or to cell components, enzyme activity or fermentation products, such as improved digestion of lactose, some immune system modulation activities, and anti-hypertensive effects. Numerous peptides with bioactive properties have been isolated from fermented dairy products. These include antibacterial, anticancer, immunomodulatory, mineral-binding, opioid and antihypertensive peptides. Viana et al. (2008) advocated that surveys involving people who identified improvements in their health -- reduction of diarrhoea, reduction of cholesterol level, among others -- after the ingestion of probiotic products, should be carried out so as to provide data on the perception and consumption of these products and the type of action of these products with respect to the population. Such studies would further enhance the awareness of medicinal values of probiotic foods. Certain probiotics have been shown to reduce the infection rate with *Clostridium difficile*, reduce the risk, severity or duration of rotavirus-induced gastroenteritis, reduce the risk of developing atopic dermatitis, or reduce the incidence of common infectious diseases. Examples of such uses for foods are numerous. Although the number of studies required to constitute convincing evidence for these uses is a subject of much discussion, the important point is that if validated, such uses for foods would be useful for consumers but often precluded by regulatory authorities (Sanders et al., 2011).

In a study on the *in vitro* angiotensin-converting enzyme (ACE)-inhibitory (ACE-I) activity of peptide fractions from different yoghurt batches (including both probiotic and normal yoghurts), inhibition of ACE activity resulted in an overall antihypertensive effect. Yoghurts were prepared

either using a sole yoghurt culture including *Lactobacillus delbrueckii* ssp. *bulgaricus* Lb1466 and *Streptococcus thermophilus* St1342; or *Lb. acidophilus* L10, *Lb. casei* L26 and *Bifidobacterium lactis* B94 in addition to yoghurt culture. All probiotic yoghurts showed appreciable ACE-I activity during initial stages of storage compared with the control yoghurt, with a significant ($p<0.05$) decrease afterwards (Donkor et al., 2007). Yoghurt produced by probiotic bacteria as adjunct culture exerted appreciable *in vitro* ACE-I activity. The development of yoghurt containing higher concentrations of released bioactive ACE inhibitors and viable probiotics may deliver health benefits to consumers.

The medicinal importance of probiotics in diarrhea treatment has been well established. The challenge of diarrhoeal diseases has been quite enormous in developing countries (Enujiugha et al., 1994) and the discovery of anti-diarrhoeal properties of probiotics has yielded remarkably positive results. With more than 1.4 million of the 9 million child deaths being attributed to diarrhoea in 2008 and 49% of them occurring in five countries namely, India, Nigeria, Democratic Republic of Congo, Pakistan and China, there has always been an urgent need for intervention to prevent and control diarrhoeal diseases. Of the various interventions, probiotics offer immense potential. The rationale for using probiotics in acute infectious diarrhoea is based on the assumption that they act against intestinal pathogens and possible mechanisms include the synthesis of antimicrobial substances, competitive inhibition of adhesion of pathogens, modification of toxin and non toxin receptors and stimulation of non specific and specific immune responses to pathogens. While research using probiotics has extended to a vast array of diseases, the most investigated field continues to remain infectious diarrhoea and compelling evidence comes from randomized placebo controlled trials. According to Hajela et al. (2010), a

recent randomized controlled trial conducted by the National Institute of Cholera and Enteric Diseases in Kolkata, India demonstrated a protective efficacy of 14% in preventing diarrhoea among children who received a probiotic. The beneficial effects of probiotics, however, are strain dependent, dose dependent, greater for doses of more than 10^{10} CFU (Figure 2), significant in people with viral gastroenteritis and more evident when treatment with probiotics is initiated early in the course of the disease (Szajewska and Mrukowicz, 2005).

Molin (2001) described a process of preparing *Lactobacillus plantarum* in cooked oat meal gruel, so as to result in about 5×10^{10} CFU/g. When administered to human patients as a rose hip fruit drink supplement, the probiotic oat gruel decreased abdominal bloating in patients with irritable bowel disease. The bacteria also increased the concentration of carboxylic acid in feces of healthy volunteers, and decreased fibrinogen concentrations in blood. The short-chain fatty acids (SCFA) such as carboxylic acid are an energy source for the mucosal cells. The reason for increased SCFA could be due to a changed composition of colonic microflora brought about by *Lactobacillus plantarum* ingestion. Another reason could also be that *Lactobacillus plantarum* was also shown, *in vitro*, to increase the amount of mucin in the colon via epithelial stimulation.

Perturbation of gut microbiota is associated with intestinal dysfunction, as illustrated during antibiotic treatment. The rise of immune disorders in developed countries, for example, is attributed to the limited exposure to harmless microorganisms. This phenomenon is called the hygiene hypothesis, and the limited stimulation of the immune system leads to such conditions as diabetes (especially in children), multiple sclerosis, allergy and inflammatory bowel disease. Specific probiotics have been shown to promote a quicker rebound from antibiotic-induced microbiota disruption (Sanders et al., 2011). In addition to the stimulating effect on the immune

system, as earlier stated above, the benefits of regular consumption of probiotics include the reduction of the risk of colon cancer, cholesterol level, incidence of diarrhea and many others. One gram of dry culture of *Lactobacillus fermentum* KC4b, for example, can remove 14.8 mg of cholesterol from culture medium (Pereira and Gibson, 2002). The anti-tumour action of probiotics is attributed to the inhibition of carcinogens and/or procarcinogens, inhibition of bacteria that convert procarcinogens to carcinogens, activation of the host's immune system and/or reduction of the intestinal pH to reduce microbial activity.

2.2 Nutritional Benefits of Probiotics

Probiotic foods not only have several potential health benefits but also have nutritional benefits (Sharma and Ghosh, 2006). Bacterial enzymatic hydrolysis has been shown to enhance the bioavailability of proteins by increasing the production of free amino acids, which can benefit the nutritional status of host particularly if the host has a deficiency in endogenous protease production. Lactic acid bacteria have also been shown to increase the content of the B-complex vitamins in fermented foods. Arora et al. (2010) investigated the effect of germination and probiotic fermentation on nutrient composition of barley based food mixtures and observed that when germinated autoclaved barley mixture was fermented with probiotic (*Lactobacillus acidophilus*) curd, it caused 14% and 11% enhancement in thiamine and niacin contents, respectively, and significant increase (34%) in lysine content. In the study, food mixtures formulated from non-germinated and germinated barley flour, whey powder and tomato pulp (2:1:1w/w) were autoclaved, cooled and fermented with 5% *Lactobacillus acidophilus* curd (10^6 CFU/g) at 37 °C for 12 h. The cell count was found to be significantly higher (8.88 log CFU/g) in the fermented food mixture formulated from germinated flour as compared to the non-

germinated barley based food mixture (7.75 log CFU/g). A significant drop in pH with corresponding increase in titratable acidity was found in the germinated barley flour-based food mixture. The combined processing caused significant improvement in reducing sugar, thiamine, niacin, lysine and soluble dietary fibre contents of barley-based food mixtures. Arora et al. (2010) concluded that a combination of germination and probiotic fermentation is a potential process for enhancing the nutritional quality of food mixtures based on coarse cereals.

The production of volatile compounds by the probiotic strain, *Lactobacillus plantarum* NCIMB 8826, in cereal-based media (oat, wheat, barley and malt) was investigated by Salmeron et al. (2009). Sixty compounds, including fatty acids and their esters, amides, alcohols, aldehydes, aromatic hydrocarbons, furans, ketones, peroxides and pyrans, were identified. *Lb. plantarum* significantly changed the aroma profile of the four cereal broths, and each substrate showed a specific volatiles profile. Oat and barley media were the substrates more influenced by the fermentation process. The most abundant volatiles detected in oat, wheat, barley and malt were oleic acid, linoleic acid, acetic acid, and 5-hydroxymethylfurfural, respectively. According to these researchers, the results obtained could contribute to the development of new non-dairy probiotic formulations. Fermentation, with probiotic organism, of indigenously developed food mixtures is a potential process for developing food products of improved nutritional quality. Food manufacturers are enthusiastic about developing such products because the added ingredients give increased value to food (Bhadoria and Mahapatra, 2011).

The fermentation process has been found to produce flavor enhancing compounds, useful enzymes and essential amino acids. It has been reported to improve the bioavailability of divalent minerals such as iron and zinc by significantly reducing the high myo-inositol phosphate

(phytate) content and other related compounds present in cereals (Enujiugha, 2006). Probiotics are associated with fermented foods. When various cereal components such as water-soluble and insoluble β-glucan and arabinoxylans, oligosaccharides, and resistant starch etc. are utilized to grow probiotic microorganisms, it is possible to realize the beneficial use of both the probiotics and prebiotic effects. The formulation of probiotics with whole grain products offers consumers both probiotics and whole grain benefits, e.g., non-digestible carbohydrates, soluble fiber, phytochemicals, and other bioactive components. When *Lactobacillus rhamnosus, Lb. plantarum,* and *Lb. lactici* strains were studied for their utilization of oat bran carbohydrates and oligosaccharides for fermentation, all three bacterial strains utilized oat β-gluco-oligosaccharides, while only *Lb. plantarum* utilized xylo-oligosaccharides. The main fermentation end products were lactic acid, acetic acid, formic acid, and ethanol (Lamsal and Faubion, 2009).

2.3 Probiotics as Functional Ingredients

A functional food can be a natural one; or foods with added components; or foods of which components have been eliminated by means of technological or biotechnological procedures. It can also be a food, in which the nature of one or more of its component has been modified, or a food in which the bio-availability of one or more of its components has been modified, or any combination of the possibilities as above (Prado et al. 2008). Regarding the functionality of probiotics, it is thought that in order to exert beneficial effects, they must be viable and available at a high concentration, typically at least 10^8--10^9 CFU per gram of product and should survive the human gastric juice in the stomach and reach the small intestine and the colon. It is generally agreed that best effect is achieved when the microorganisms colonize the intestinal epithelium

since they can affect the intestinal immune system, displace enteric pathogens, and provide antimutagens and antioxidants, and possibly other effects by cell signaling.

As pointed out earlier, the selection of a probiotic strain for human health and nutritional benefits requires that several aspects of functionality have to be considered: 1. Acid tolerance and tolerance to human gastric juice. 2. Bile tolerance (an important property for survival in the small bowel). 3. Adherence to epithelial surfaces and persistence in the human GI-tract. 4. Immunostimulation, but no proinflammatory effect. 5. Antagonistic activity against pathogens such as *Helicobacter pylori, Salmonella* sp., *Listeria monocytogenes* and *Clostridium difficile*. 6. Antimutagenic and antigarcinogenic properties. The adhesion to mucosal surfaces by probiotic microorganisms is an important ability for the colonization of the human gastrointestinal tract, prevents their elimination by peristalsis and provides a competitive advantage over pathogens. Adhesion provides an interaction with the mucosal surface facilitating the contact with gut associated lymphoid tissue mediating local and systemic immune effects. Thus, only adherent probiotics have been thought to effectively induce immune effects and to stabilise the intestinal mucosal barrier. Auto-aggregation appeared to be necessary for the adhesion of probiotic strains to intestinal epithelial cells and co-aggregation abilities may form a barrier that prevents colonization by pathogens (Collado, 2008). The adhesion process can be divided into two steps: i.e. reversible adhesion due to long-range forces, and subsequent interaction mediating a direct contact between microorganisms and supports surfaces such as the hydrophobic interaction of microorganism and support. Biopsy sampling probably gives the most accurate information on the adhesion ability of probiotic strains. However, there are severe limitations in the technique: first and most importantly, ethical considerations limit the use of the technique. Secondly, the

technique is very laborious and therefore only few individuals can be included in trials. Thirdly, the evacuation of the colon prior to colonoscopy probably leads to a loss of large number of adhering bacteria, leaving only the bacteria with the strongest adhering ability attached (Saarela et al., 2000). It could be argued that strong adhesion ability may increase the risk of infection in the host. Also, some probiotic strains are poorly adhering *in vitro* and/or *in vivo* and still they can show positive effects in the hosts.

The antimicrobial properties of probiotics can be attributed to both the competition for nutrients and the production of inhibitory compounds such as organic acids, hydrogen peroxide and bacteriocins. The lowering of pH due to organic acids (especially lactic and acetic acids) produced by these bacteria in the gut has a bactericidal or bacteriostatic effect (Ukeyima et al., 2010; Shah, 2007). The capacity to produce different antimicrobial compounds may be one of the critical characteristics for effective competitive exclusion of pathogen survival in the intestine and expression of a probiotic effect for the host. Although probiotic strains may produce bacteriocins, their role in the pathogen inhibition *in vivo* can only be limited, since traditional bacteriocins have an inhibitory effect only against closely related species such as other *Lactobacillus* or on spore-fomers such as *Bacillus* or *Clostridium*. However, low molecular weight metabolites (such as hydrogen peroxide, lactic and acetic acid, and other aroma compounds) and secondary metabolites may be more important since they show wide inhibitory spectrum against many harmful organism like *Salmonella*, *Escherichia coli*, *Clostridium*, and *Helicobacter*. Lactic acid bacteria of the genera *Lactococcus*, *Pediococcus* and *Lactobacillus* produce diacetyl which is rarely present in food fermentations at sufficient levels to make a major contribution to antibacterial activity (Ukeyima, 2010). However, diacetyl production in

sufficient amount inhibits the proliferation of food pathogens. The efficacy of bacteriocins particularly nisin to inhibit target organisms in food is determined by the chemical composition and physical conditions of the food system. The inhibition of such bacterial cells is caused by destabilization of the function of the cytoplasmic membrane.

3.0 CEREAL-BASED PROBIOTIC FOODS

Milk and its fermentation products, such as yogurts, have been the probiotic carriers of choice for various reasons, including milk's recognition as a healthy product. However, more recent research efforts into probiotic potentials of cereal-based beverages are yielding remarkable results. There are a wide variety of traditional non-dairy fermented beverages produced around the world, and many of them are non-alcoholic beverages manufactured with cereals as principal raw material (Prado et al. 2008). According to Lamsal and Faubion (2009), cereal and cereal component-based foods offer opportunities to include probiotics, prebiotics, and fiber in human diet. Cereals contain water-soluble fiber (such as β-glucan and arabinoxylan), oligosaccharides (such as galacto- and fructo-oligosaccharides) and resistant starch, and thus have been suggested to fulfill the prebiotic concept. Whole grains are also sources of many phytochemicals, including phytoestrogens, phenolic compounds, antioxidants, phytic acid and sterols (Katina et al., 2007). Fermentation of cereals provides optimum pH conditions for enzymatic degradation of phytate and releases minerals such as manganese (which is an important growth factor of LAB), iron, zinc and calcium. Strains of *Lactobacillus* have been recognized as complex microorganisms that require fermentable carbohydrates, amino acids, B-group vitamins, nucleic acids and minerals to grow, and therefore fermentation of cereals may represent a cheap way to obtain a rich substrate that sustains the growth of beneficial microorganisms.

Increasingly, whole grain matrix is becoming one of the favored choices as a probiotics delivery vehicle. This is mainly because the formulation of probiotics with whole grain products offers consumers both probiotics and afore-mentioned whole grain benefits, e.g., non-digestible carbohydrates, soluble fiber, phytochemicals, and other bioactive components (Lamsal and Faubion, 2009). This not only enhances the dietary value of the product as a whole, but also appeals to an emerging consumer lifestyle. While fermented dairy foods have been conventionally associated with probiotics, cereal-based products have been developed mainly for the combined effect of probiotics, prebiotics, and dietary fibers. As the bran and germ supply the majority of the biologically active components found in grains, whole grain cereals and cereal components could serve as probiotic carriers with the combined advantages of healthful bioactive components and fibers. Oats and barley are the cereals with highest content of β-glucan, recognized as the main functional component of the cereal fibers. Studies have indicated the hypocholesterolemic effect of this compound, leading to 20--30% reduction of LDL-cholesterol, and to an expected overall effect of reduced cardiovascular disease risk.

3.1 Fermented Cereal-Based Products

Fermented maize product, ogi, is a popular weaning and breakfast cereal in sub-Saharan Africa. Ogi is traditionally prepared by natural fermentation (steeping maize grains in water for 2-4 days at room temperature), followed by wet milling, sieving and souring of slurry (2-3 days rest at room temperature) (Enujiugha, 2006). The periods of fermentation and souring determine the degree of sourness (measured by titratable acidity) and, to a large extent, the nutrient status of ogi. The predominant microorganisms in ogi fermentation include *Lactobacillus plantarum, Lb. fermentum* and *Streptococcus lactis*, among other LAB species (Table 2). In many parts of

Nigeria, nursing mothers do give their babies ogi liquor (water from the fermented cereal pulp) and this causes the termination of such illness as diarrhea and abdominal discomfort (Ukeyima et al., 2010). Adebolu et al. (2007) evaluated the antibacterial activities of ogi liquor from different grains against some common diarrhoeal bacteria in southwest Nigeria and discovered the inhibition of the pathogens by the ogi liquor which contains a variety of organisms including *Lactobacillus* species. This and other animal-based *in vivo* studies point to the ogi fermentative organisms as having probiotic qualities (Oyetayo and Osho, 2004). Ogi is usually consumed as a hot semi-solid gruel called akamu, or a steamed pudding called agidi; in both forms the heat treatment destroys the lactic acid bacteria present, and therefore its probiotic effects. However, the souring water is normally packaged and sold as a sweetened rich probiotic beverage and encouraged by nutritionists as a health-promoting drink and anti-diarrhoeal tonic.

Gowé is an indigenous fermented sorghum-based sour beverage, which is widely consumed in urban areas of Benin Republic. It is made from a blend of malted and non-malted sorghum flour that is produced by spontaneous fermentation involving mixed cultures of lactic acid bacteria (LAB) and yeasts. The fermentation process takes place in an environment with a moisture content varying between 52 and 87%. The sweet and sour dough obtained by decantation during fermentation needs to be cooked and further diluted in water to obtain the beverage. The dominant microorganisms of gowé fermentation were the lactic acid bacteria *Lactobacillus fermentum, Weissella confusa, Lactobacillus mucosae* and *Pediococcus acidilactici*, and the yeasts *Kluyveromyces marxianus and Pichia anomala*. Some of the lactic acid bacteria mentioned above have been proven to exhibit probiotic properties. Current efforts are geared towards the utilization of the sweet and sour dough without the hydrothermal treatment in order

to preserve the probitoic qualities. A significant decrease in pH from 6.1 to 3.3, with a concomitant increase in titratable acidity (11 to 60 g/kg as lactic acid, dry weight), was observed after 24 h of fermentation when LAB was used either alone or in combination with yeasts in controlled conditions (Vieira-Dalodé et al., 2008). The LAB count increased significantly from 6.1 to 9.4 log CFU/ml, while the yeast count remained constant throughout fermentation.

Prado et al. (2008) mentioned Bushera as the most common traditional beverage consumed by both children and adults in the Western highlands of Uganda. The product is a common delight both in the urban and rural areas of Western Uganda. The sorghum, 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). Studies on the probiotic potentials of the fermentative organisms are still inconclusive, but evidence of anti-diarrhoeal properties are established.

Borde is a cereal-based traditional fermented beverage and is widely consumed in the southern and western parts of Ethiopia. It is produced by spontaneous fermentation using rudimentary equipment (Abegaz et al., 2002). Borde is an opaque, effervescent, whitish-grey to brown coloured beverage, with a dark consistency and a sweet-sour taste. It is an important product because both adults and children often consume it as a low cost meal replacement. Maize is more frequently used than other cereals (wheat, tef, sorghum, finger millet and barley) for borde fermentation because of its low price (Abegaz et al., 2002) in rural Ethiopian villages. A

combination of lactobacilli and yeasts are known to be involved in the traditional fermentation of a mixture of malted cereals (75%) and unmalted cereals (25%) for borde production. It usually takes about 4 days to produce a batch of borde. An average worker consumes about 1 to 2 litres of borde a day, which could sustain the individual without additional food for most of the day (Abegaz et al., 2002). Consumers believe that borde enhances lactation and mothers are encouraged to drink substantial amounts of it after giving birth. Borde is also considered to alleviate malaria, diarrhea, constipation and abscesses.

Gadaga et al. (1999) reported on mahewu, a sour beverage made from corn meal and sorghum/millet malt, which is commonly fermented by *Lactococcus lactis* subsp. *lactis*. It is prepared from the maize porridge, which is mixed with the water. The sorghum, millet malt, or wheat flour is then added and left to ferment. The spontaneous fermentation process is carried out by the natural flora of the malt at the ambient temperature. *Lactobacillus bulgaricus* and *Lactobacillus brevis* have also been isolated from mahewu. Kwete is a Ugandan fermented cereal-based beverage mainly produced from a mixture of maize and malted millet flour. The millet grains are the only raw materials which are soaked (24-48 h), germinated (2-3 days) and sun dried (1-2 days) during kwete production. The process of souring to produce sourdough is uncontrolled and carried out at ambient temperatures (24-30 °C) for 24 h. Kwete is ready for consumption within 24 h of fermentation and after being filtered using a cheese cloth or a bag woven from grass (Namugumya and Muyanja, 2009). Filtering gives the kwete a smooth mouthfeel as well as contributes to its uniform colour distribution. Good quality kwete is creamish to light brown in colour, with a thick consistency and a sweet-sour taste. The *Lactobacillus* and *Lactococcus* counts ranged from 5.40 to 7.36 log CFU/ml during fermentation.

The greatest increase in lactic acid bacteria was noted between 24 and 48 h. Higher numbers of *Lactobacillus* than *Lactococcus* were observed, though by end of 72 h fermentation both species attained the same microbial population. Fermentation of kwete is a spontaneous process initiated by the lactic acid bacteria and yeasts from both the malt and roasted sourdough. Malt also contributes the fermentable sugars and enzymes that initiate the fermentation process (Namugumya and Muyanja, 2009).

Kunun-zaki is a fermented non-alcoholic cereal beverage whose popularity in Nigeria is due to its characteristic sweet-sour taste typical of other lactic acid bacterial fermented foods of African origin such as mahewu and baganiya (Efiuvwevwere and Akoma, 1995). The traditional processing of kunun-zaki involves the steeping of millet or sorghum grains, wet-milling with spices (ginger and pepper), wet-sieving and partial gelatinization of the slurry, followed by the addition of sugar and bottling. A brief fermentation usually occurs during kunun-zaki processing. This short fermentation which usually takes place during steeping of the grains in water over a period of 8-48 h, is known to involve both lactic acid and yeasts. Three species of lactic acid bacteria (*Lactobacillus plantarum, Lb. fermentum* and *Lactococcus lactis*) were isolated from fermenting kunun-zaki and characterized by Agarry et al. (2010)

Angelov et al. (2006) produced a symbiotic functional drink from oats by combining a probiotic starter culture and whole-grain oat substrate. The substrate was fermented with *Lb. plantarum* B28. The levels of starter culture concentration, oat flour and sucrose content were established for completing a controlled fermentation for 8 h. The addition of aspartame, sodium cyclamate, saccharine and Huxol (12% cyclamate and 1.2% saccharine) had no effect on the dynamics of the fermentation process and on the viability of the starter culture during the product

storage. The β-glucan content in the drink of 0.31--0.36% remained unchanged throughout fermentation and storage of the drink. The viable cells counts reached at the end of the process were about 7.5×10^{10} CFU/ml. The shelf life of the oat drink was estimated to 21 days under refrigerated storage.

Another probiotic food, togwa is a starch-saccharified beverage made from maize flour and finger millet malt (Prado et al. 2008) and usually consumed in the southern part of Tanzania. It is consumed by the working people and also used as refreshment as well as a weaning food. In the production of togwa, cereal flour is cooked in the water. After cooling at 35°C, seed culture (old togwa) and cereal flour from the germinated grains are added. The fermentation process finishes at pH 4.0-3.2 (Molin, 2001). In a study on the microbiological and fermentation characteristics of togwa, Mugula et al. (2003) observed that the process was predominated by lactic acid bacteria (LAB) and yeasts. The isolated microorganisms were identified as *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus cellobiosus, Pediococcus pentosaceus, Weissella confusa, Issatchenkia orientalis, Saccharomyces cerevisiae, Candida pelliculosa* and *Candida tropicalis*. The pH decreased from 5.24--5.52 to 3.10--3.34. Maltose increased initially and then decreased, fructose decreased and glucose levels increased during the first 12 h of fermentation. The organic acids detected during fermentation included dl-lactic, succinic, formic, pyruvic, citric, pyroglutamic and uric acid. Lactate was the predominant acid and increased significantly with time. The volatile organic compounds (VOC) detected included acetaldehyde, 2-methyl-propanal, 2-methyl-butanal, 3-methyl-butanal, ethanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, diacetyl and acetoin.

Pozol is a refreshing beverage, consumed in the Southeastern Mexico, made by cooking maize in an approximately 1% (w/v) 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 ambient temperature for 0.5--4 days. The fermented dough is suspended in the water and drunk (Prado et al. 2008). Some fibrous components are not completely solubilized by nixtamalization and sediment is present in the beverage when the dough is suspended in the water. Mageu is a sour, non-alcoholic beverage popular among the indigenous people of Southern Africa, including farmers, school children and mine workers. It is consumed as a refreshing drink as well as a weaning food. Mageu is prepared by boiling maize flour in water to make porridge of 8 to 10% solids (w/w) to which wheat flour is added to initiate the lactic acid fermentation at 35°C for 24 h (Byaruhanga et al., 1999). The lactic acid bacteria involved in mageu fermentation did not produce bacteriocins antagonistic to *Bacillus cereus*. The production of lactic acid and its pH-lowering effect were the major inhibitory factors. Thus, lactic acid production by the starter culture should be considered a critical factor in ensuring the safety of mageu. The major microorganism involved in mageu fermentation wasidentified as *Lactobacillus lactis* (Byaruhanga et al., 1999).

Boza, which is a low-pH and low alcohol beverage produced in the Balkan Peninsula with wheat and other cereals has been described as a natural source of probiotic LAB by Todorov et al. (2008). Boza has become a very popular beverage consumed daily by people of all ages due to its pleasant taste, flavour and high nutritional values. 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*. Strains of *Lb. fermentum, Lb. pentosus, Lb. paracasei* and *Lb. rhamnosus* isolated from boza produced bacteriocins active against *Lb. casei, Escherichia coli, Pseudomonas aeruginosa* and *Enterococcus faecalis*. No plasmids were recorded, suggesting that the genes encoding the bacteriocins are located on the genomes. Strains of *Lb. fermentum* and *Lb. plantarum* exhibited antimicrobial activity and inhibited the growth of *Klebsiella pneumonia* by the production of bacteriocins in a study carried out by Von Mollendorf et al. (2006). During lactic acid fermentation of Boza, *Bacillus cereus*, a known pathogen commonly found in cereals, is inhibited by the acidity developed to pH 2.6 and 0.8% titrable acidity after 72 h. No strains of LAB were found to produce bacteriocins agaist *B. cereus*.

Saman et al. (2011) investigated the growth on rice-based media of the probiotic strain *Lactobacillus plantarum* NCIMB 8826 isolated from the human gut and found that rice bran or rice bran extracts could be used in new probiotic food developments, while probably still maintaining other functional properties of the bran. Fermentation broths were obtained from the whole grain brown rice and rice bran of two Thai rice cultivars, RD6 (glutinous) and RD17 (non-glutinous). The rice used was not germinated and fermentations were carried out in a single step without growth supplementation. *Lb. plantarum* grew well in all tested broths, and a final biomass value of approx. 10.4 log CFU/mL was obtained in 30 h. The results confirmed that brown rice and rice bran are suitable substrates for the culture of the probiotic *Lb. plantarum* NCIMB 8826. Rice, the major cereal in Asia, and its products could be an economical and

beneficial medium to develop probiotic foods. Another method to enhance their quality of nutrients and bioactive compounds is germination, and since this process increases the content in proteins, amino acids, sugars and vitamins, it has been proposed for the growth of probiotic bacteria (Trachoo et al., 2006). The growth of four probiotic bacteria (*Lb. acidophilus, Lb. pentosus, Lb. plantarum* and *Lb. fermentum*) was studied in germinated rough rice powder (5%, w/w) mixed with water. This was compared to medium regular rice powder and NaCl was also added. *Lb. plantarum* exhibited the highest ($p<0.05$) growth in the medium containing germinated rough rice powder with and without NaCl, and *Lb. acidophilus* showed the lowest growth.

Sobia is a traditional cereal-based fermented beverage, which is popular and has been prepared for many years in the Western region of Saudi Arabia. It is sold after the end of the fermentation time (24 h) and is usually consumed cold (Gassem, 2002). Traditionally, sobia is a sweet-sour beverage (pH 3.44-4.0) prepared from malt and wheat flour which are suspended in water. Sugar and spices (cardamon and cinnamon) are added to the mixture and usually left to chance inoculation from the environment in a warm place. Preliminary results revealed that sobia samples had high total bacterial and lactic acid bacteria counts that ranged from 4.17 to 8.09 log CFU/ml and from 4.01 to 8.19 log CFU/ml respectively. Yeasts counts ranged from 3.96 to 5.87 log CFU/ml. Lactic acid bacteria consisted of *Lactobacillus cellobiosus* (26.8%), *Lb. buchneri* (17.9%), *Lb. plantarum* (8.9%), *Lb. brevis* (23.2%), *Lb. delbrueckii* subsp. *delbrueckii* (8.9%), *Leuconostoc lactis* (8.9%),and *Pediococcus pentosaceus* (5.4%). Yeasts comprised *Saccharomyces cerevisiae* (27.6%), *Candida tropicalis* (26.3%), *C. ciferrii* (13.1%), *C. guilliermondii* (13.1%), *C. lipolytica* (6.58%), *Kloeckera japonica* (13.1%),and *Rhodotorula*

rubra (1.3%) (Gassem, 2002). The process of *sobia* production involves suspending malt flour (150-200 g) and wheat flour (150-200 g) in water (8-10 l). Sugar (7-10%) and spices (cardamon and cinnamon) are added to the mixture and usually left to chance inoculation from the environment in a warm place (30-40 °C) for 24 h.

3.2 Process Optimization and Characterization

Charalampopoulos et al. (2002a) have done experiments with different cereals to determine the main parameters that need to be considered in the growth of probiotic microorganisms, defining them as follows: the composition and processing of cereal grains, the substrate formulation, the growth capability and productivity of the starter culture, the stability of the probiotic strain during storage, the organoleptic properties and the nutritional value of the final product. The intensity of the interactions between probiotics and both the food matrix and the starter organisms depends in large part on the time that probiotics are added to the product, that is, whether they are present during fermentation or are added after. In the latter case, interactions may be minimal because addition may occur immediately before or even after cooling below 8°C and the metabolic activity of starters and probiotics is drastically reduced at these temperatures (Heller, 2001). When probiotic bacteria participate actively in fermentation, the aspects of food composition and of interactions with the food matrix and starters have to be taken into account on a much larger scale.

Currently, industrial demand for new technologies that enable high cell yield at large scale and ensure probiotic stability in food remains strong, because many strains of intestinal origin are difficult to propagate and high survival is important for both economic reasons and health effects (Lacroix and Yildirim, 2007). In addition, more efficient technologies could lead to greater

product efficacy and strain diversification and this could result in the transformation of technologically 'unsuitable' strains into useful products. Processes that include sublethal stress applications during cell production and new fermentation technologies, such as immobilized cell biofilm-type fermentations could be used to profile cell physiology to optimize survival and functionality in the gut. Figure 3 shows some of the factors affecting the viability of probiotics from production to the gastrointestinal tract. Compositions of the growth medium and final cell mass are important in monitoring cell survivality. A pioneer work on gowé by Vieira-Dalodé et al. (2008) shows that an accelerated fermentation was obtained when *Lb. fermentum* was used individually or in combination with *K. marxianus* during the controlled fermentation of a mixed malted and non-malted sorghum flour for gowé production. Gowé obtained within 7 h of controlled fermentation was judged to be similar to the product obtained by spontaneous fermentation by sensory evaluation. This improved process required a 2 h saccharification of the malted flour at 40°C instead of 12 h of primary fermentation at 30°C. Such an improvement constitutes important progress in the traditional technology of gowé production.

Several approaches have been investigated to enhance cell viability during downstream processing, storage and, eventually, digestion. These include the application of sublethal stresses during fermentation, the addition of protectants, including compatible solutes (e.g. betaine which has been extensively studied), and cell protection by microencapsulation (Lacroix and Yildirim, 2007). The survival of *Bifidobacterium longum* in growth medium at 6°C significantly increased when cells were submitted to starving conditions for 30 or 60 min (Maus and Ingham, 2003). Likewise the acid tolerance of *Bifidobacterium lactis* (at pH 3.5 in synthetic gastric fluid) increased significantly after decreasing the growth medium pH from 6.0 to 5.2 and under

conditions of starvation. In a patented process (Lamsal and Faubion, 2009), lactic acid bacteria (LAB) were grown in a culture medium made from a hot-water (60°C) extract of cereal germ. While the cereal grain from which germ came was not specifically identified in their process, rice and wheat germs were mentioned as possible sources. The cereal germ extraction process was aided by the presence of up to 5% w/w of a starch hydrolyzing enzyme which provided sugar to support bacterial growth. The medium (10--20% solids w/w) prepared with the germ extract was inoculated with LAB and cultured. The resulting broth was claimed to be suitable for consumption on its own after some flavoring, or as a fortifying nutritive supplement after drying. The bacterial population was also reported to increase 'exceedingly' when amylases were added during extraction. Simulation studies have also helped to establish cereal components, especially high fiber β-glucan containing cereals, as effective bacterial growth enhancers.

The ability of microorganisms to grow and survive depends largely on their capacity to adapt to changing environments. Adaptation to adverse environments is usually associated with the induction of a large number of genes, the synthesis of stress-response proteins, and the development of cross-resistance to various stresses. On the basis of this knowledge, methods for enhancing the long-term survival of Lactobacilli and Bifidobacteria by decreasing the lethal effects of environmental stresses and process conditions have been recently investigated to improve cell resistance to environmental stresses occurring during production, storage or digestion. Oat bran powder slurry at 2.7 g/L was fermented with *Lactobacillus*- CG, among others, and fed to a simulator of the human intestinal microbial ecosystem along with other nutrients (Lamsal and Faubion, 2009). The effect of fermented oat-bran feeding was evaluated by analyzing bacterial population, short-chain fatty acid and gas production. Results showed that

the bacterium *Lactobacillus*- CG colonized the simulator reactor for several weeks, persisting in the reactor even through the interval between the feeding periods. This suggested that a saturation level was reached in the prebiotic substrate beyond which there is no further change in bacterial activity in the system. Oat bran feeding also favored the growth of *Bifidobacteria* in the simulator.

The immobilization and growth of cells in porous solid supports during incubation in nutritive medium results in the formation of a high cell density region (cell concentrations typically ranging from 5×10^{10} to 5×10^{11} CFU/ml, which are 10- to 50-fold higher than for traditional batch cultures). For food applications, cell entrapment in food grade biopolymer gel matrices (e.g. κ-carrageenan, alginate and gellan) has been most widely used. Several advantages over free-cell fermentations have been demonstrated: high cell densities, reuse of biocatalysts, improved resistance to contamination and bacteriophage attack, enhancement of plasmid stability, prevention from washing-out during continuous cultures, and the physical and chemical protection of cells. For example, continuous fermentation with *Bifidobacterium longum* immobilized in gellan gum gel beads produced high cell concentrations and four-fold increased volumetric productivity at a dilution rate of 0.5 h^{-1} when compared with free-cell batch cultures at optimal pH (Doleyres et al., 2002).

3.3 Involvement of Probiotics as the Fermentative Organisms

It is important to state that while some of the fermentative organisms identified in the popular traditional cereal-based beverages as lactic acid bacteria have not been established as probiotic, the majority of the organisms have exhibited probiotic qualities in randomized animal assays (Rivera-Espinoza and Gallardo-Navarro, 2010). Probiotic organisms are expected to possess the

following characteristics: (1) Easy reproducibility; (2) Ability to survive the environmental conditions of the location where they are active; (3) Genetically stable without plasmid transfer; (4) The absence of allergic, toxic, mutagenic or carcinogenic reactions, with neither its fermentation products nor its cell components being deleterious after consumption by the host; (5) Ability to remain viable during processing; and (6) Ability to adhere to and colonize the location where they are active (Ukeyima, 2010). *Lactobacillus acidophilus* has been considered the predominant lactobacilli in the intestinal tract of healthy humans, and therefore is the organism most commonly used in probiotic products. Its growth occurs at a temperature as high as 45°C; however, the optimum is found between 35 and 40°C. The organism grows in slightly acidic media at pH of 6.4-4.5, but growth will stop at a pH of 4.0-3.6 (Rivera-Espinoza and Gallardo-Navarro, 2010). This bacterium tolerates from 0.3% to 1.9% titrable acidity, with an optimum pH at 5.5-6.0. Invariably, *Lb acidophilus* was not mentioned as a starter in some of the cereal-based traditional beverages, although other members of the LAB family are prominent.

In selecting starter micro-organisms reliable acid-forming ability is the most important characteristic. However, when selecting probiotics the criteria should be connected to the impact on human health and well-being. As the environment within the gastrointestinal tract and within the food might be quite different the probiotic is often not suitable as a starter organism. The growth rate might be too slow and they might give off-flavours. This could partly be overcome by using specific aseptic processes as used in producing fermented acidophilus milk with levels of probiotics reaching 10^9 CFU/g. Another possibility is to improve the suitability of the food as a substrate for the probiotic by adding energy sources (e.g. glucose), growth factors (e.g. yeast extract and protein hydrolysates) or suitable antioxidants, minerals or vitamins. However, even if

such an adjustment may improve the performance of the probiotic as a starter, it is often not enough (Saarela et al., 2000). By use of a help starter in addition to a probiotic preparation this problem can usually be solved. When producing probiotic-containing fermented products a fermentation temperature of 37-40°C is usually recommended since these temperatures span the range in which most probiotic strains multiply well. As most probiotics grow well at 37°C a thermophilic starter might be preferable to a mesophilic one.

In recognition of the importance of assuring safety, even among a group of bacteria that is generally recognized as safe, it has been recommended (Pineiro and Stanton, 2007), that probiotic strains be characterized at a minimum with the following tests: 1) determination of antibiotic resistance patterns, 2) assessment of certain metabolic activities (d-lactate production, bile salt deconjugation), 3) assessment of side effects in humans, 4) epidemiological surveillance of adverse incidents in consumers, 5) testing for toxin production (if the strain under investigation belongs to a species that is a known mammalian toxin producer), and 6) testing for hemolytic activity if the strain under evaluation belongs to a specie with known hemolytic potential. Lactic acid bacteria 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. Examples of *Lactobacillus* spp involved in lactic acid bacteria fermentation of cereal based fermented foods include *Lb. plantarum, Lb. casei, Lb. sakei, Lb. acidophilus,* and *Lb. salivarius* among others. Kalui et al. (2010) reported the isolation of *Lb. fermentum, Pediococcus pentosaceus, Lb. plantarum, Weisella confusa* and *Lb. rhamnosus* from ikii, a traditional fermented maize porridge (the liquor is commonly consumed as sweetened beverage) in Kenya.

In this product, *Lb. fermentum* was the predominant species (43% of isolates), followed by *Pediococcus pentosaceus* which formed 38% of the isolates; *Lb. plantarum* formed 10%, *Lb. confusa* formed 8% and least was *Lb. rhamnosus* which formed 1% of the total isolates.

Lb. fermentum and *Lb. plantarum* have been reported to be the most commonly associated lactic acid bacteria species with spontaneous lactic acid fermentations of cereal products. In their studies of koko, Lei and Jakobsen (2004) reported levels of *P. pentosaceus* ranging from 8.3 to 33.3%. *Lactobacillus confusa* has been isolated in low numbers from mawe, togwa, wheat sourdough and bushera. These researchers reported isolation of *Lactobacillus fermentum, Pediococcus pentosaceus, Lactobacillus confusa* and *Lactobacillus paraplantarum* from koko, a fermented millet product. It has been demonstrated that LAB and yeasts are the most abundant microorganisms in spontaneous fermentation that produces sour products. These microorganisms are the most predominant microorganisms involved in the fermentation process for Ogi production. *Lb. plantarum, Lb. fermentum, Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* have been identified in ogi production using different cereal grains (Ijabadeniyi, 2007). There appears to be a beneficial relationship existing between LAB organisms and yeast in these fermentations. In the work of Gassem (2002), different microorganisms were isolated and identified from sobia samples. The lactic acid bacteria that were isolated and characterized belonged to three major genera, *Lactobacillus, Pediococcus*,and *Leuconostoc*. Among the 112 lactic acid bacteria isolated, 86% (96 isolates) were *Lactobacillus* spp. whereas 8.9% (10 isolates) were *Leuconostoc*, and 5.4% (six isolates) *Pediococcus*. *Lactobacillus* spp. were identified as *Lactobacillus cellobiosus* 30 isolates (26.8%), *Lb. buchneri* 20 isolates (17.9%), *Lb. plantarum* 10 isolates (8.9%), *Lb. brevis* 26 isolates (23.3%), and *Lb. delbrueckii* subsp.

delbrueckii 10 isolates (8.9%). *Leuconostoc* spp. was identified as *Leuconostoc lactis* 10 isolates (8.9%) and *Pediococcus* spp. as *Pediococcus pentosaceus* 6 isolates (5.4%).

3.4 Probiotic Qualities and Functionality of the Cereal-based Beverages

Roberfroid (2000) noted that among the most promising targets for functional foods are the gastrointestinal functions, including those that control transit time, bowel habits, and mucosal motility as well as those that modulate epithelial cell proliferation. Promising targets are also gastrointestinal functions that are associated with a balanced colonic microflora, that are associated with control of nutrient bioavailability (ions in particular), that modify gastrointestinal immune activity, or that are mediated by the endocrine activity of the gastrointestinal system. Moreover, some systemic functions such as lipid homeostasis that are indirectly influenced by nutrient digestion or fermentation represent promising targets. Indeed, the colon's fermentation capacity may be modified after probiotic intake, and oral intake of certain lactic acid bacteria will increase the number of lactobacilli or bifidobacteria in human faeces.

The possible utilization of cereal constituents in probiotic food formulations could be as fermentable substrates for starter cultures (e.g., lactic acid bacteria), encapsulation material, and/or dietary fiber supplement. When various cereal components such as water-soluble and insoluble β-glucan and arabinoxylans, oligosaccharides, and resistant starch etc. are utilized to grow probiotic microorganisms, it is possible to realize the combined beneficial qualities and potentials of both the probiotics and prebiotic (Lamsal and Faubion, 2009). Although *in vitro* studies have been reported in great number, *in vivo* studies involving cereals or cereal components as probiotic growth medium and/or feed in animals and humans are scarce. It has already been emphasized that to achieve health benefits, probiotic bacteria should be viable in

the range of about 10^6-10^7 CFU/g of product during (i.e. at the point of) consumption and sustain this viability during transit in the gastrointestinal tract. This functionality is enhanced by probiotic encapsulation using appropriate prebiotics. However, how this functional aspect could be achieved in traditional beverages without any encapsulation is yet to be established.

Ross et al. (2005) noted that the most important aspect of the functionality of probiotic cultures is their ability to promote human health at the site of action. However, prior to achieving this, the cultures must be ingested and survive gastric transit in sufficient numbers to elicit their effects. Given that most intestinal isolates can be difficult to cultivate *in vitro*, the efficient delivery of live cultures represents a major challenge in probiotic product development. Some of the novel approaches currently employed to improve the survival of probiotic strains during processing in food systems and following ingestion range from encapsulation of the probiotic strains in prebiotic substances (synbiotics) to genetic manipulation. Traditional approaches involve, but not limited to, increasing the inoculum size to such levels that the required minimum number would still be maintained at the site of action. Alternatively, the use of natural variants via application of the principle of *survival of the fittest* could yield strains that can adapt to the harsh processing and GIT conditions. *Bifidobacterium bifidum* cells encapsulated in gel beads composed of alginate, pectin, and whey proteins, and surrounded by two membranes exhibited good survival at pH 2.5 for up to 2 h, while free cells did not survive under these conditions, and furthermore protection was also afforded by this system, when the cells were exposed to bile salt solutions. Furthermore, it was found that encapsulating lactobacilli in calcium-alginate beads improved their heat tolerance. Adopting a genetic manipulation approach to the improvement of probiotic cultures also has potential in increasing their viability when exposed to stressful

environments such as those encountered during functional food development or during gastric transit. There has been a dramatic increase in sequence information for intestinal lactobacilli and bifidobacteria which has recently culminated in the availability of entire genome sequence data for a number of strains including *Lactobacillus plantarum*, *Lactobacillus johnsonii* and *Bifidobacterium longum* while incomplete genome information is available for many other human intestinal isolates. The use of housekeeping genes as taxonomic markers holds great promise in the development of standardized and globally accessible methods for the identification of LAB used in the food industry. It has been shown that the fingerprinting techniques, Amplified Fragment Length Polymorphism (AFLP) and repetitive DNA element-PCR (rep-PCR), allow unequivocal differentiation of *Lactobacillus* and *Bifidobacterium* species more than the 16S rDNA sequences (Vankerckhoven et al. 2008).

The probiotic strain must show high functionality in the following areas: oxygen tolerance, acid tolerance, bile tolerance, heat tolerance, ability to grow in the food substrate and ability to effectively metabolize prebiotics. In addition, from a food processing point of view, probiotic strains should be suitable for large-scale industrial production (Bhadoria and Mahapatra, 2011) with the ability to survive under both food processing conditions and storage; and the presence of the probiotic culture in the food product should not adversely affect product quality or sensory properties. The use of oxygen-impermeable containers, microencapsulation, incorporation of nutrients, and selection of stress-resistant strains are applied to address these issues. Modern researches in genomics and proteomics have made it possible to identify the genes involved in *Lactobacillus* stress responses, such as the molecular chaperone groESL and dnaK genes for heat

stress, methionine sulfoxide reductase genes for oxygen stress, and F_1F_0-ATPase genes for acid stress.

3.5Storage Stability of Probiotic Products

The viability of probiotics is a key parameter for developing probiotic foods. The ability of microorganisms to grow and survive depends largely on their capacity to adapt to changing environments. Adaptation to adverse environments is usually associated with the induction of a large number of genes, the synthesis of stress-response proteins, and the development of cross-resistance to various stresses. On the basis of this knowledge, methods for enhancing the long-term survival of lactobacilli and bifidobacteria by decreasing the lethal effects of environmental stresses and process conditions have been recently investigated to improve cell resistance to environmental stresses occurring during production, storage or digestion (Lacroix and Yildirim, 2007). A probiotic must exhibit high survival rates in downstream processes (such as centrifugation and drying) and in food products during storage. High survival through the upper gastrointestinal tract and high viability at its site of action are also a requirement, together with high activity in the gut environment. Figure 3 outlines the factors that affect the viability of probiotic bacteria until they reach the target site of the host.

The temperature stress that the microorganism may undergo during processing and storage and its effect on bacterial survival is an important consideration. Most studies showed that higher survival rates of lactic acid bacteria were obtained at lower storage temperatures. Saxelin et al. (1999) reported that the survival of *Lactobacillus* strain at -18°C was poor, showing a decrease of 1-log units in cell count. At -35°C, however, the organism's viability improved, with a decreased cell count to 0 to <1 log unit. They were fully stable at -45°C with no losses. Storage

at room temperature, which is common for several types of non-dairy products such as cereal products and drinks, can create an overwhelming challenge for probiotic stability. This problem can sometimes be solved by using probiotic encapsulation technology to ensure the viability and stability of probiotic cultures. The evaluation of cell viabilities in stored probiotic foods is of vital importance economically and technologically, and it is also important for efficacy. In addition, the reliable determination of viability is a prerequisite to the much-needed regulation of and legislation on the quality of probiotic products. A subpopulation of non-culturable cells retained a functional cell membrane typical of viable cells, indicating that probiotic bacteria become dormant during storage (Lahtinen et al., 2005). This could be exploited in enhancing the viability of the fermentative organisms in traditional beverages during transit to retail outlets and before actual consumption.

The packaging materials used and the storage conditions under which the products are kept are important for the quality of products containing probiotic bacteria (Saarela et al., 2000). Foods used for dissemination of probiotics are usually fermented foods. Fermented foods are produced by a microbial fermentation in which fermentable carbohydrates are transformed into ethanol and/or organic acids mainly acetic, lactic and propionic acids. In fermented probiotic products it is important that the probiotic culture used contributes to good sensory properties. Therefore it is quite common to use probiotic bacteria mixed together with other types of bacteria suited for the fermentation of the specific product.

Spray-dried powder harbouring high numbers of viable probiotics is a convenient means of storage and transport of probiotic cultures and their subsequent application in functional foods. While spray drying is an economical process for the large-scale preparation of these

cultures, and is commonly used for the preparation of food ingredients, it suffers from the disadvantage of causing bacterial cell injury and death, which has been attributed primarily to the effects of heat and dehydration leading to destruction of the properties and performance characteristics of probiotic cultures. One approach used by a number of workers to improve probiotic performance in food systems is the addition of protectants to the media prior to drying. For example, the incorporation of thermoprotectants such as trehalose, non-fat milk solids and/or adonitol, growth-promoting factors, including various probiotic/ prebiotic combinations and granular starch have been employed in efforts to improve culture viability during drying, storage and/or gastric transit.

4.0 FUTURE RESEARCH FOCUS

Two areas of interest are currently being explored for the enhancement of probiotic potentials of traditional LAB-fermented cereal-based beverages, namely, the development of stress-tolerant starters with proven probiotic qualities and the sustenance of the probiotic attributes both at the point of consumption and through the GIT up to the site of action in the colon. Interestingly, almost all the traditional cereal-based beverages that are consumed in the third world are products of natural inoculation and seed culture technique via back-slopping. Much research efforts are needed towards upgrading (from controlled laboratory fermentations to pilot and industrial scales) and standardization of the traditional fermentations (Enujiugha et al., 2008). The introduction of microencapsulated probiotic cultures would be a welcome development, although local superstitious beliefs and the idea of natural foods could hinder such novel techniques.

The incorporation of probiotics into other traditional non-cereal-based beverages is currently being examined as a way of helping the rural populations solve worsening health conditions. Ukeyima et al. (2010) observed that the diverse plant based beverages consumed by the indigenous peoples could be used as potential carriers for probiotic bacteria. For example, there are different formulated beverages from ginger, sobo (from *Hibiscus sabdariffa* (roselle) calyx) (Awe et al., 2013) and peanut containing appropriate quantities of probiotic organisms with remarkable shelf stability under tropical ambient conditions. The problem that must be addressed for the smooth introduction of such drinks into local diets is the widespread ignorance regarding probiotics. Presently, the knowledge of the health and nutritional benefits of probiotic foods are inadequate even among clinicians and food processors in Nigeria and other developing countries of the world. In a survey involving the use of close-ended and open-ended structured questionnaires and 125 randomly sampled medical practitioners, 95.2% of the respondents were not familiar with the term probiotics, and all (100%) indicated that they would like more information on the subject (Anukam et al., 2006). Up to 75% of the participants raised some concerns bordering on the safety and receptivity of probiotic products among the prospective users. However, the awareness is gradually building up with the recent interest on medicinal foods by the diverse populations, especially rural dwellers. This has led to increased consumption of fermented cereal-based beverages in these areas. However, the use of unfermented beverages as probiotic vehicles is still at the laboratory experimentation level, and none has, at present, been commercialized. Such drinks are expected to be embraced in the near future.

A relatively unexplored area of research is the incorporation of probiotics into fruit juices. Some pioneer laboratory works in this regard are currently receiving worldwide attention. For example, Pereira et al. (2011) optimized the conditions of *Lactobacillus casei* NRRL B-442 cultivation in cashew apple juice, during refrigerated storage (4°C) for 42 days. The optimum conditions for probiotic cashew apple juice production were initial pH 6.4, fermentation temperature of 30°C, inoculation level of 7.48 Log CFU/ml (*Lb. casei*) and 16 h of fermentation process. Viable cell counts were higher than 8.00 Log CFU/ml throughout the storage period (42 days). Cashew apple juice showed to be as efficient as dairy products for *Lb. casei* growth. Correa et al. (2008) investigated the effect of probiotic cultures on sensory performance of coconut flan during storage at 5°C and the viability of these microorganisms for up to 28 days. Sensory analyses of the product were performed after 7, 14 and 21 days of storage. Coconut flans were produced with no addition of cultures (control), or supplemented with *Bifidobacterium lactis*, *Lactobacillus paracasei* and *Bf. lactis* + *Lb. paracasei*. Populations of *Lb. paracasei* and *Bf. lactis* as single or in co-culture remained above 7 log CFU/g during the entire storage period. These workers concluded that, viability of both *Lb. paracasei* and *Bf. lactis* in coconut flans were always above the recommended levels of 10^6-10^7 CFU/g during refrigerated storage, thus satisfying this criterion established for a probiotic food. The addition of *Lb. paracasei* and *Bf. lactis* to coconut flan therefore resulted in a product with great potential as a functional food, and with excellent sensory features. It is expected that in the near future, a greater percentage of tropical fruits will be evaluated for their potentials as probiotic delivery vehicles.

Another area that needs careful study is probiotic incorporation alongside the application of legume supplementation/enrichment of cereal-based beverages for tackling the prevailing

protein-energy malnutrition in the third world. For example, ogi is produced from maize, which is limiting in lysine and tryptophan. It is now common practice to supplement ogi with a legume protein, especially soybeans and other locally available legumes, for amino acid complementarities (Enujiugha, 2006; Oluwamukomi et al., 2005). It is important to establish to what extent legume incorporation into cereal-based beverages would influence their capacity to sustain probiotics, and their widespread acceptance among consuming populations.

5.0 CONCLUSIONS

This paper has reviewed the potentials of LAB-fermented cereal-based beverages as probiotic delivery vehicles. The incorporation of probiotic bacteria with scientifically supported health claims in foods has great potentials for improving the quality of life. The ability to produce bacteriocins is often discussed as a desirable property of probiotics; however, antagonism to starter cultures and vice versa may be a limiting factor for combinations of starters and probiotics. The establishment of probiotic LAB starters would definitely provide a solution to this problem. In this light, it should be emphasized that the various cereal-based probiotic beverages consumed in different parts of the world could be seen as overcoming the starter-antagonism challenge, since the starters are majorly lactic acid bacteria strains. Most of these strains have proven clinical records as known probiotics. Added to this, the cereal components serve as excellent prebiotics for the sustainability of the probiotics during production, storage and consumption. In conclusion, the intricate and complex involvement of rural farmers and processors in the production of probiotic beverages requires more detailed scientific observation/examination, especially with regard to the strict adherence to standard hygienic practices which such processes demand. Education and enlightenment of local processors and

consumers (made up largely of rural populations), coupled with the upgrading and optimization of local technologies would go a long way to sustain the health and functional benefits of such cereal-based probiotic beverages in the developing countries. If the awareness of the medicinal and nutritional benefits of consuming probiotics is properly disseminated to local processors, it is expected that extra care would be taken to sustain probiotic functionality in the products.

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Table 1: Conditions, organisms involved and examples of probiotic foods

Conditions for a food product	-Must have live organisms ≥ 10^6 CFU/ml
to be classified as probiotic	-Organisms are members of LAB family
	-The organisms are resistant to gastric acidity and bile salts
	-No negative nutritional effects on the human body
Microgorganisms used as probiotics	-*Lactobacillus casei, Lb. acidophilus, Lb. brevis, Lb. lactis*
	Lb. plantarum, Lb. fermentum, Lb. delbrueckii var. bulgaricus
	-*Bifidobacterium breve, Bf animalis, Bf. lactis, Bf. bifidum,*
	Bf. longum, Bf. adolescentis
	-other organisms (*Lactococcus lactis, Enterococcus faecium,*
	Enterococcus faecalis, Pediococcus acidolactici,
	Streptococcus sali var. thermophilus, Saccharomyces boulardi)
Examples of probiotic foods	-Dairy-based foods: yoghurt, cheese, nunu, etc
	-Non-dairy-based foods: ogi souring water, fufu liquor,
	fermented raffia palm sap, etc.

Derived from Ukeyima et al., 2010

Table 2: LAB-fermented cereal based traditional African beverages

Beverage	Substrate	Country of origin	Fermentative organisms
Ogi, Akamu	Maize, Sorghum, Millet	Nigeria	*Lactobacillus plantarum, Streptococcus lactis, Lactobacillus fermentum*
Gowe	Sorghum	Benin Republic	*Lactobacillus fermentum, Weissell confusa, Lb. mucosae, Pediococcus acidilactici*
Busher a	Sorghum, Millet	Uganda	*Lb. brevis, Streptococcus lactis, Leuconostoc mesenteroides, Lactococcus lactis*
Mahew u	Corn meal, Sorghum/Millet malt	Zimbabwe	*Lactococcus lactis, Lb. bulgaricus, Lb. brevis*
Kunun-Zaki	Millet, Sorghum	Nigeria	*Lb. plantarum, Lb. fermentum, Lactococcus lactis*
Togwa	Maize, Finger millet	Tanzania	*Lb. plantarum, Lb. brevis, Lb. fermentum, Lb. cellobiosus, Weissella confusa*
Mageu	Maize, Wheat	South Africa	*Lb. lactis, Lb. fermentum*
Borde	Maize, finger millet, tef	Ethiopia	*Lb. acidophilus, Lb. fermentum*
Kwete	Maize, Millet	Uganda	*Lactobacillus brevis, Lb. plantarum, Lactobacillus lactis*
Koko	Millet	Kenya	*Lb. confusa, Pediococcus pentasaceus, Lb. fermentum, Lb. paraplantarum*

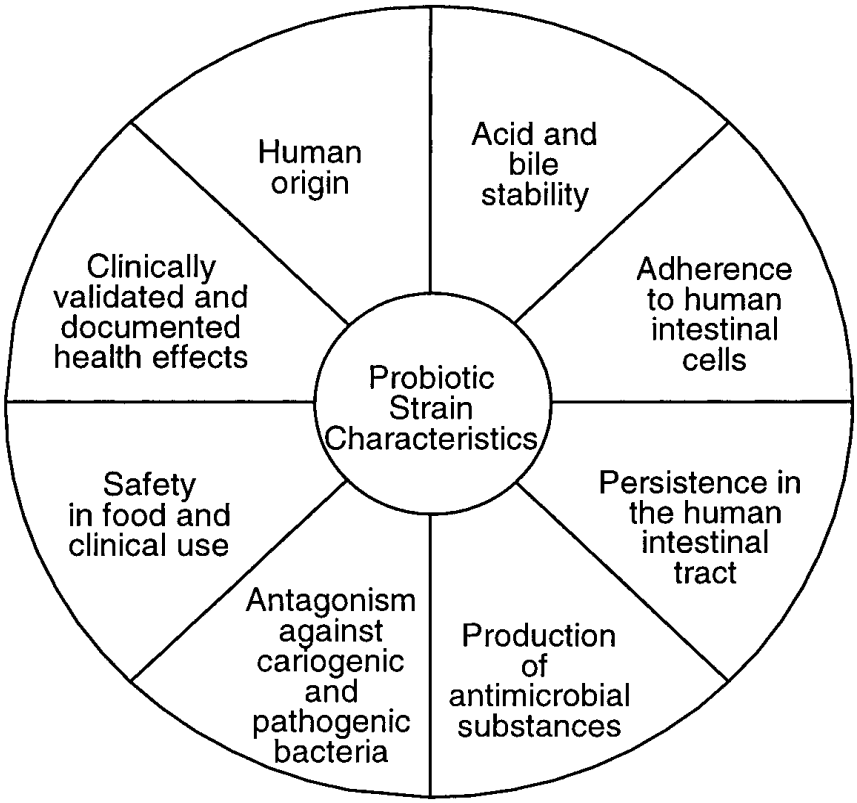

Figure 1. The theoretical basis for selection of probiotic microorganisms

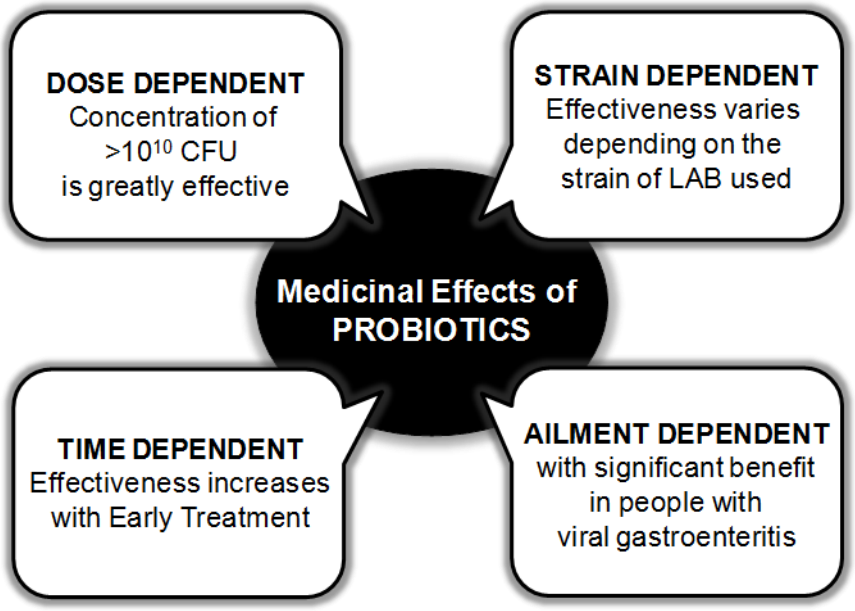

Figure 2. The medicinal effects of probiotics are dependent on many factors

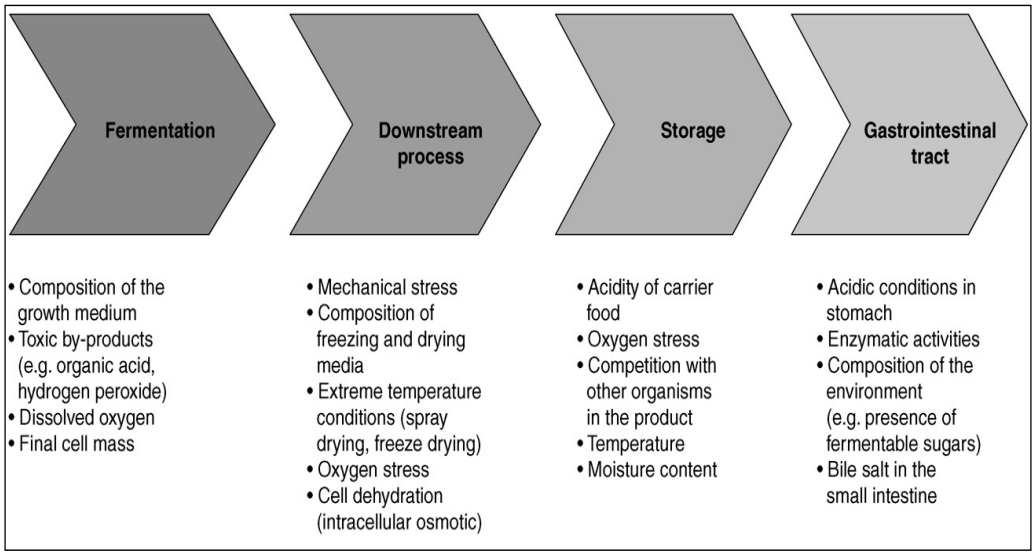

Figure 3. Factors affecting the viability of probiotics from production to the gastrointestinal tract.