Review

Current applications of probiotic foods in Africa

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Currently, there is a growing interest in the consumption of probiotic foods due to their reported health benefits. In developed countries, probiotics have been extensively studied and this has led to the production of a variety of probiotic foods especially with dairy milk. However, the use of beverages from plant materials as a potential carrier of probiotic microorganisms is receiving increasing attention. Various indigenous fermented foods containing probiotic bacteria have been part of local diet in Africa due to reported medicinal properties they possess. However, the usual challenge confronting the commercialization of the probiotic beverage drinks in Africa is the safety assessment of probiotic strains due to lack of requisite facilities as well as technical manpower. This review therefore, captures local fermented drinks that are usually consumed as probiotic foods by Africans.

Key words: Probiotics, functional foods, non dairy sources, safety assessment, African applications.

INTRODUCTION

The term probiotics was introduced by Lilly and Stillwell in 1965 to describe growth-promoting factors produced by microorganisms. Probiotic is derived from Greek and means pro-life (Havenear and Veld, 1992). 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, 2001; Tomasik and Tomasik. 2003). Probiotics are selected from the strains most beneficial for the host intestinal bacteria. Members of the genera Lactobacillus, Bifidobacterium and the yeast Saccharomyces boulardii are mainly used (Oyetayo and Osho, 2004; Miles, 2007; Prado et al., 2008). Other organisms are also applied, such as members of the genera Lactococcus and Enterococcus (Mercenier et al., 2003). Lactobacilli are considered as indigenous microorganisms colonizing the small intestine as they are found within the first week of life (Salminen et al., 1996). The prophylactic and therapeutic effects of probiotic micro-organisms are known to commonly

include:

- (a) Balancing the intestinal flora;
- (b) Increasing lactose tolerance and ingestion;
- (c) Reducing cholesterol levels;
- (d) Synthesis of B complex vitamins;
- (e) Absorption of calcium;
- (f) Modulating the immunological system.

These are essentially obtainable only when the organisms are consumed in the range of 10^6 cfu/ml and above (Table 1).

The gastrointestinal tract contains millions of bacteria which are exposed to daily challenges that can cause an imbalance between the so-called 'healthy' and pathogenic bacteria (Miles, 2007). Factors like starvation, heat, stress and poor nutrition have been shown to alter the composition of the gut microflora (Tannock, 1983). The distortions in microbial ecosystems with loss of indigenous microorganisms imply deregulation of autogenic factors and vacated habitats. Such conditions could be exploited by commensal or transient micro-organisms and if they are potentially pathogenic, the outbreak of an opportunistic infectious disease is possible (Havenear and Veld, 1992).

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Conditions for a food product to be classified as probiotic	 Must have live organisms ≥ 10⁶ cfu / ml Organisms are members of LAB family The organisms are resistant to gastric acidity and bile salts No negative nutritional effects on the human body 	
Microorganisms used as probiotics	 Lactobacillus casei, L. acidophilus, L. brevis, L. lactis, L. plantarum, L. fermentum, L. delbrueckii var. bulgaricus Bifidobacterium breve, Bf. animalis, Bf. Lactis, Bf. bifidum, Bf. longum, Bf. adolescentis Other organisms (Lactococcus lactis, Enterococcus faecium, Enterococcuc faecalis, Pediococcus acidolactici, Streptococcus salivarus var. thermophilus, Saccharomyces boulardi) 	
Examples of probiotic foods	 Dairy-based foods: yoghurt, cheese, nunu Non-dairy-based foods: ogi souring water, fufu liquor, fermented raffia palm sap 	

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

Elie Metchnikoff (1845-1916) 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 (Havenaar and Veld, 1992; Tomasik and Tomasik, 2003; Molin, 2007). This assertion later prompted the international launch of yoghurt and until recently, milk has been the main carrier of Probiotics (Molin, 2007).

Probiotics are usually administered through carriers like ice cream, cheese, yoghurt, break fast cereals and capsules containing freeze-dried cell powders (Zhang, 2004). Fuller (1992) reported that probiotics can also be presented in the form of liquid suspensions, granules and pastes. Sometimes probiotics are consumed together with prebiotics. Prebiotics are defined as non digestible substances (dietary fiber) that exert some biological effect on humans by selective stimulation of growth or bioactivity of beneficial microorganisms either present or therapeutically introduced to the intestine. The microflora concerned are the probiotic organisms and the prebiotics are sources of energy for probiotics (Gibson and Roberfioid, 1995; Tomasik and Tomasik, 2003).

The consumption of probiotic products actively affects the growth of other bacteria because they lower pH levels and produce bacteriocins which equally inhibit the survival of pathogens in the gastrointestinal inflammation (Molin, 2007). Other beneficial effects of probiotics include their anticholesterolaemic effect (Schrezenmeir and Vrese, 2001), antitumour effect, lactose hydrolysis and immune modulating response (Hosono et al., 1992; Ouwehard et al., 2002).

In Africa, the concept of probiotics and the production of probiotic foods are equally gaining acceptability, especially with the knowledge that some traditional African foods containing beneficial bacteria (lactic acid bacteria) have been an acceptable diet among the local people. This paper therefore, highlights the applications of probiotic foods in Africa and the nutrient potentials that could be utilized via the production of probiotic foods using locally available plant materials.

PROBIOTIC ORGANISMS

The use of lactic acid bacteria (LAB) as probiotics for human and animal consumption has been well documented (Yoon et al., 2004; Savadogo et al., 2006). *Lactobacillus* and *Bifidobacteria* species are the microorganisms which are most commonly used as human probiotics (Table 1). In particular, strains of *Bifidobacterium animalis*, *Bifidobacterium lactis*, *Bifidobacterium bifidum*, *Bifidobacterium breve* and *Bifidobacterium longum* biotypes *infantis* and *longum* are often implemented in probiotic products in combination with other lactic acid bacteria (Masco et al., 2005). Among the *Lactobacillus* species, *Lactobacillus casei* is the most widely studied, because of its effects on the reduction of incidence and duration of various types of diarrhea and its capacity to modulate the immune system.

Bifidobacteria are classified under thegenus Bifidobacterium which is included in the family Actinomycetacea (Stackebraudt et al., 1983). They are commonly isolated from feaces of humans, animals, birds and are present in high numbers in breastfed babies (Mitsuoka, 1992). Bifidobacteria are morphologically similar to some Lactobacillus species and were previously included in the genus Lactobacillus. The cells are grampositive rods of various shapes and sizes, present as single cells or in chains of different sizes. They are nonspore forming, non-motile and anaerobic, although some can tolerate oxygen in the presence of carbon dioxide (Ray, 2004). Bifidobacteria share many metabolic properties of LAB such as being fermentative and producing lactate, among other acids and are commonly included in this group in many discussions on probiotics (Vankerckhoven et al., 2008).

Lactobacilli are rods, usually long and slender that form

chains in most species (Frazier and Westhoff, 2002). They are gram-positive, non-spore forming, aerotolerant anaerobes which lack cytochromes and porphyrins and are therefore, catalase and oxidase negative. Some do take up oxygen through the mediation of flavo-protein oxidase and/or re-oxidize NADH produced during the dehydrogenation of sugars (Adams and Moss, 1999).

Lactobacilli are divided into 3 main groups based on their metabolic pathways and fermentation and products. These groups include:

Group I

These are homofermentative utilizing the Embden Meyerhof-Parnas (EMP) pathway to produce lactic acid. Organisms in this group include *L. acidophilus* and *L. delbrueckii.*

Group II

These are facultative heterofermenters producing lactic acid or a mixture of lactic acid, acetic acid, ethanol and formic acid. This group contains several organisms like *L. plantarum, L. casei, L. Coryniformis, L. Curvatus* and *L. farciminis.*

Group III

They are obligate heterofermentative organisms, producing lactic acid, acetic acid, carbon dioxide and possibly ethanol. They are capable of growth at a wide range of temperatures (2 - 53°C) but with optimum growth at 30-40°C. This group consists of *L. brevis*, *L. buchneri*, *L. bifermentans*, *L. fermentum*, *L. cellobiosus* and *L. viridescens* (Brookes and Buckle, 1992).

Lactic acid bacteria used as probiotics are identified using various biochemical, phenotypic and genotypic techniques. The use of biochemical systems like API 50CH and API 20 STREP is not encouraged to be the sole methods of identification. Although such preliminary procedures could be used at the genus level in conjuncttion with primary phenotypic tests, multiple DNA- based methods should be employed (Vankerckhoven et al., 2008). Molecular pattern-based techniques such as pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD), polymerase chain reaction (PCR), ribotyping and protein based methods such as SDS-PAGE and DNA-DNA homology (Mitzuoka, 1992; Vankerchkhoven et al., 2008; Kos et al., 2008) are usually employed. However, the use of 16S rDNA sequence analysis is commonly recommended for LAB analysis.

Specialized equipment, trained personnel and modern

databases with dependable reference materials are needed for a successful molecular identification of probiotic organisms. However, the absence of these requirements could be overcome by getting typed cultures from international public culture collection centers like American type culture collection (ATCC), national collection of industrial bacteria (NCIB, Scotland), national collection of type cultures (NCTC, England) and collection of the institute pasteur (CIP, France) (National Academy of Sciences, 1979).

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 plamid 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 (Havenaar and Veld, 1992; Wolfgang et al., 1999; Sejong et al., 2000).

METABOLITES FROM PROBIOTICS

The antibiosis of lactic acid bacteria has been attributed to fermentation products and bacterial enzymes. The antibiosis due to fermentation products include organic acids, oxidation-reduction (OR) potential, bacteriocins and antibiotic substances (Custy et al., 1992).

Organic acids which include lactic, acetic and propionic acids do exhibit antibacterial property due to the lowering of pH of the medium. The lower pH of the medium interferes with the maintenance of membrane potential and inhibits active transport (De vugst and Vandamme, 1994) and may be mediated both by dissociated and undissociated acid (Cherrington et al., 1991). The antagonistic property of each of these acids against unwanted microorganisms at a given molar concentration is not equal. Acetic acid has a higher inhibitory profile than lactic acid and can inhibit yeasts, moulds and bacteria (Blom and Mortvedt, 1991). Acetic and propionic acids have higher pKa values than lactic acid and therefore, have a higher undissociated ratio than lactic acid at a given pH. This difference may be one reason for their increased antimicrobial efficacy compared to lactic acid (Earnshaw, 1992).

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. However, diacetyl production in sufficient amount inhibits the proliferation of food pathogens (Caplice and Fitzgerald, 1999).

Different genera and species of Lactic acid bacteria producing bacteriocins have been isolated. Some of the species are *Lactococcus lactis*, *Streptococcus themophilus*, *Lactobacillus plantarum*, *L. acidophilus* and *L. bulgaricus* (Ray, 1992). Bacteriocins are proteinaceous anti-bacterial compounds, which constitute a heterologous subgroup of ribosomally synthesized antimicrobial peptides (De Vugst and Vandamme, 1994). Chemically, bacteriocin peptides are cationic, amphipathic, have α helical or β -sheet structures or both and can have thioesthers, disulfide bridges, or free thiol groups (Ray, 2004). Bacteriocins display hydrophobic properties and the bacterial membrane is in most cases the target for their activity (Savadogo et al., 2006).

Hurst (1981) referred to bacteriocins as "biological food preservatives" since bacteriocins, unlike antibiotics, are not used for medicinal purposes. Gram-positive bacteria like lactic acid bacteria produce bacteriocins small in size within the range of 3-6 KDa (Nes et al., 1996) but there are some that are larger in size (Klaenhammer, 1993). Majority of the gram-positive bacteriocins are active compounds that increase the permeability of the cytoplasmic membrane (Jack et al., 1995).

Majority of the bacteriocins are active at low pH (Garcia -Garcera et al., 1993). There are some strains of organisms that may naturally produce more than one type of bacteriocin (Dodd and Gasson, 1994). Genetic modification of bacterial strains has led to the heterologous expression of bacteriocins (Rauch et al., 1994; Allison et al., 1995). Bacteriocins are normally destroyed by digestive enzymes unlike classical antibiotics (Caplice and Fitzgerald, 1999).

According to the procedure proposed by Klaenhamner (1993), bacteriocins are divided into 4 major groups. Many of the bacteriocins produced by bacteria associated with food belong to classes I and II. Class I bacteriocins are called lantibiotics and contain post-translationally modified amino acids. Generally, class I bacteriocins usually contain 19 to over 50 amino acids and is subdivided into class Ia and class Ib (Altena et al., 2000). Class II bacteriocins contain small heat-stable, non-lantibiotics, non-modified peptides and can be subdivided into Class IIa, IIb and IIc. On the other hand, class III bacteriocins are large and heat stable while class IV bacteriocins is composed of an undefined mixture of proteins, lipids and carbohydrates (Klaenhamner, 1993; Chikindas et al., 2001).

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 (Jack et al., 1995; Chikindas et al., 2001).

The synthesis of bacteriocins in lactic acid bacteria is

growth associated and usually occurs throughout the growth phase and ceases at the end of the exponential phase or probably before the end of growth (Lejeune et al., 1998). Bacteriocins production is influenced by the type and amount of carbon, nitrogen, phosphates, cations, surfactants and inhibitors (Savadogo et al., 2006).

Bacteriocins often have synergies with other treatments (like temperature, water activity (a_w) , pH, E_h and presservatives) and can be used as a hurdle to improve the safety of food. An understanding of the mode of each individual hurdle therefore, allows the most effective combination of treatments.

PREBIOTICS AND SYNBIOTICS/EUBIOTICS

Prebiotics are non-digestible substances (dietary fiber) that exert some biological effect on humans by selective stimulation of growth or bioactivity of beneficial microorganisms either present or therapeutically introduced to the intestine. According to Gibson et al. (2004), prebiotics are selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health. Prebiotics undergo fermentation by beneficial microflora in the large intestine. The microflora concerned are the probiotic organisms and the prebiotics are sources of energy for probiotics (Gibson and Roberfroid, 1995; Tomasik and Tomasik, 2003). Prebiotics are oligosaccharides extracted from natural sources (e.g. inulin and oligofructose (OFS)) or synthesized from disaccharides (e.g. transgalacto-oligosaccharides) (Table 2). The most commonly studied of the prebiotics include inulin and OFS which are found in many vegetables, including onions, asparagus and chicory root (Dysseler and Hoffem, 1995).

Prebiotics stimulate useful microorganisms; promote immunodulation and bacteriostatic activity which might result from blocking receptors open for interaction with pathogenic bacteria (Tomasik and Tomasik, 2003). Commercially available prebiotics include: Galacto-oligosaccharides (GOS), soybean oligosaccharides (SOS), isomalto-oligosaccharides (IMO), xylo-oligosaccharides (XOS), glucooligosaccharides, fructooligosaccharides, lactulose, inulin-type fructans and lactosucrose (LS) (Playne and Crittenden, 1996; Wang, 2009).

Enzymatic processes are utilized for the commercial manufacture of prebiotics and this could either be by building up the desired oligosaccharide from readily available sugars by the process of transglycosylation or by the hydrolysis of large polysaccharides (Nakakuki, 1993).

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 by selectively stimulating the growth and/or by activating the

Classification criteria	•	resistant to the upper gut tract
	•	fermentation by intestinal microbiota
	•	beneficial to the host's health
	•	selective stimulation of probiotics
	•	stability to food processing treatment
Human prebiotic	•	Inulin-type fructans
ingredients	•	Oligosaccharides of xylose, glucose,
		fructose and galactose
	•	Lactulose, lactosucrose
	•	soybean oligosaccharides
	•	Isomaltooligosaccharides
Some functional properties	•	Fat or sugar replacement
of prebiotics	•	Moisture retention
	•	Foam stabilization
	•	Fibre
	•	Improved mouthfeel / texture

Table 2. Classification criteria and food applications of prebiotics.

Adapted from Wang (2009).

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 (Tomasik and Tomasik, 2003).

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 (Ray, 2004).

FUNCTIONAL FOOD AND PROBIOSIS

Functional food may be considered as a therapeutic aid available without prescription. With functional food, various functions of organisms can be modulated. Functional food not only prevents various diseases but also protects from effects of environment pollution (Tomasik and Tomasik, 2003). Yoghurt is a classical example of a functional food with probiotics. Yoghurt with probiotics, called bio-yoghurt, should contain living bacterial cultures. There is a growing market potential for functional foods because they are believed by consumers to enhance their health and well-being (Hillian, 1995).

Japan is the birth place of the term functional food (Kubomara, 1998) and this was developed during the 1980s but the concept gained legal status in 1991, being described as FOSHU, or "foods for specified health use" (Prado et al., 2008). Foods identified as FOSHU are required to provide evidence that the final product, but not isolated individual component(s), is likely to exert a health

or physiological effect when consumed as part of an ordinary diet (Roberfroid, 2000).

A variety of terms, more or less related to the Japanese FOSHU, has appeared worldwide. In addition to functional foods, these include more exotic terms such as nutraceuticals, designer foods, medifoods, vitafoods, therapeutic foods and prescriptive foods (Finley, 1996; Roberfroid, 2000).

A functional food can be a natural one; or foods added of components; or foods of which components have been eliminated by means of technological or biotechnological procedures. It also can 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).

Nutraceuticals are specific chemical compounds in food, including vitamins and additives, that may aid in preventing disease or food products transformed into powder, pills, syrups, drinks and other medicinal forms not generally associated with food and demonstrated to have physiological benefits or provide protection against chronic disease (Defelice, 1995; Polk, 1996).

Dietary supplements are products designed to supplement the diet and that contains one or more of certain specified dietary ingredients (vitamins, minerals, herbs or other botanicals and amino-acids) to supplement the diet by increasing total dietary intake, a concentrate, extract or combination (Gibson et al., 2000).

Initially, functional foods were produced with the addition of components like vitamins and mineral elements like iron and calcium due to their health benefits (Ziemer and Gibson, 1998). Functional food has several definitions which include: foods or food products marketed with the massage of the benefit to health (Riemersma, 1996), food derived from naturally occurring substances that can and should be consumed as part of the daily diet and that serve to regulate or otherwise affect a particular body process when ingested (Smith et al., 1996). They are food similar in appearance to conventional food, which is consumed as part of a usual diet and has demonstrated physiological benefit and/or reduces the risk of chronic disease beyond basic nutritional functions (Health Canada, 1997).

Probiotic bacteria are sold mainly in fermented foods and dairy products play a predominant role as carriers of probiotics. These foods are well suited to promoting the positive health image of probiotic (Heller, 2001).

APPLICATIONS OF PROBIOTICS IN AFRICA

Due to the inexpensive nature of fermentation technology, fermented beverages have made an important contribution to the human diet in many African countries. Several fermented dairy products are known to be produced at the artisan level in different African countries. Kindirmo, nono and warankasi are common fermented milk products in Nigeria. Kindirmo is prepared by fermenting cow milk with overnight portion of previously prepared kindirmo which is reported to contain strains of lactic acid bacteria. The milk is normally heated, allowed to cool and fermented in a big calabash or any suitable container at ambient temperature for a period of 8 h. The curdled kindirmo is then homogenized by stirring and sweetened to taste. Nono is the fermented skimmed milk that is prepared by the same procedure like kindirmo. Warankasi is known among indigenous African consumers as cheese just as kindirmo and nono are considered as the equivalent of yoghurt. Warankasi is a dairy based product that is fermented by the artisans using an overnight portion of warankasi which have been reported to contain mainly Lactococcus, Streptococcus and Lactobacillus strains of lactic acid bacteria which ferment the heated milk within a period of 8 - 10 h. Alternatively, a plant known as Sodom apple (Calotropis procera) could be used (Belewu et al., 2005). This plant is reported to contain an enzyme Calotropin which curdle the milk into cheese.

In Zimbabwe, a traditional fermented milk known as *amasi* or *zifa* is consumed by the people. This product is produced by leaving fresh raw bovin milk to ferment spontaneously at ambient temperature in earthenware pots or plastic containers. The fermentation process occurs within 24-72 h depending on the temperature of the ambient environment. Strains of lactic acid bacteria like *Enterococcus*, *Lactococcus* and *Lactobacillus* have been implicated to be responsible for the fermentation

reaction (Gadaga et al., 1999).

In many parts of Nigeria, nursing mothers do give their babies' ogi liquor (water from fermented cereal pulp) and this causes the termination of their illness. 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.

Cereals and cereal components can be used as fermentation substrates for probiotic organisms imparting prebiotic effects (Lamsal and Faubion, 2009; Zhibing, 2004). This not only enhances the dietary value of the product as a whole, but also appeals to an emerging consumer lifestyle. Prado et al. (2008) mentioned Bushera as the most common traditional beverage consumed by both children and adults in the Western highlands of Uganda. This beverage is produced by fermenting germinated cereal flours in water for 1 - 6 days. with Lactobacillus brevis as the most commonly isolated microorganism. 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. Another probiotic food, Togwa is a starch-saccharified beverage made from maize flour and finger millet malt (Prado et al., 2008).

Currently, there is an increasing demand for probiotic products of non-dairy materials (Shah, 2001). As such, 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 and peanut containing appropriate quantities of probiotic organisms.

CONCLUSION AND FUTURE AREAS OF RESEARCH

The increasing public awareness about the health promoting properties of probiotic foods is a commendable development that should be promoted. In America, Europe and oriental countries particularly Japan where there exist the trained manpower and available technologies for commercial production of various types of probiotic (both dairy and non-dairy) foods, consumers normally access from a variety of such foods.

In Africa, there is also a growing awareness about the health promoting properties of probiotic foods and peoples of different regions in Africa produce foods containing probiotic organisms, though at small scale level. Therefore, there is the need to train manpower and develop technologies that would optimize production processes beginning from reliable characterization of the probiotic organisms to strain level. An exploitation of nondairy materials as potential carriers of the probiotic organisms would assuredly be a viable alternative to consumers who are either lactose intolerant or vegetarians.

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