African fermented foods and probiotics

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1. Introduction
1.1. Probiotics, definition and suggested health-improving properties
1.2. A global perspective on the use of probiotics
1.3. Why Africa needs probiotics

2. An overview of African fermented foods
2.1. Non-alcoholic cereal and vegetable fermentations
2.1.1. Examples of African fermented maize products
2.1.2. Examples of fermented sorghum
2.1.3. Examples of fermented millet
2.2. Starchy root crop fermentations
2.3. Animal protein fermentations

3. Choice of multifunctional strains for African probiotics
3.1. The search for potential probiotic strains to supplement African fermented foods
3.2. Intervention studies with African potential probiotics

4. Further perspectives

5. Recommendations and research needs
5.1. Choice of multifunctional strains
5.2. Choice of product for probiotic delivery

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1. Introduction

1.1. Probiotics, definition and suggested health-improving properties

Kollath (1953) and Vergio (1954) were probably the first to introduce the term probiotic (Holzapfel and Schillinger, 2002), while a beneficial association of lactic acid bacteria (LAB) with the human host was already suggested in Biblical times and by Metchnikoff (1908). The latter considered the longevity of Bulgarian peasants to be related to their high intake of fermented milk products, as he considered gut microbes detrimental rather than beneficial to human health (Metchnikoff, 1908). While documentation of said longevity is scant, recent large studies in Scandinavia support at least the ability of regular intake of fermented foods to reduce the incidence of serious disease (Larsson et al., 2008; Keszei et al., 2010; Sonestedt et al., 2011). In this context the LAB and their production of lactic acid as a result of sugar metabolism were suggested to be health-promoting agents. Originally defined as microorganisms promoting the growth of other microbes (Vergio, 1954; Lilly and Stillwell, 1965), probiotics have been re-defined a number of times. In 1989, Fuller referred to the animal host when he defined a probiotic as ‘a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance’ (Fuller, 1989). Havenaar et al. (1992) defined probiotics as ‘mono- or mixed cultures of live microorganisms which, when applied to animal or man, beneficially affect the host by improving the property of the indigenous flora’, while in relation to food, probiotics were considered as ‘viable preparations in foods or dietary supplements to improve the health of humans and animals’ (Salminen et al., 1998). The German Federal Institute for Health and Consumer Protection and Veterinary Medicine (currently the Federal Institute for Risk Assessment) defined probiotics as ‘specific live microorganisms which reach the intestinal tract in active form and in sufficient numbers to positively affect the health of the host’ (Franz et al., 2011a). Currently the most common definition is that from the FAO/WHO which states that probiotics are ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’ (FAO/WHO, 2002).

The mechanisms of health-improving properties of probiotics are still not completely understood, but are commonly suggested to relate to pathogen interference, exclusion or antagonism, immunomodulation, anticarcinogenic and antimutagenic activities, alleviation of lactose intolerance symptoms, reduction in serum cholesterol levels, reduction in blood pressure, prevention and decreasing incidence and duration of diarrhea, prevention of bacterial vaginosis and urinary tract infection, maintenance of mucosal integrity, and improved peridontal health (Erbringer et al., 1995; Reid et al., 1995; Salminen et al., 1996; Kleahnhammer and Kullen, 1999; Burns and Rowland, 2000; de Vrese et al., 2000; Saarela et al., 2000; Holzapfel and Schillinger, 2002; Ouwehand et al., 2002; Isolauri, 2004; Hummelen et al., 2010; Gupta, 2011; Kumari et al., 2011). Many studies, which were also subsequently evaluated by meta-analyses, have clearly shown that positive effects can be observed with specific strains, especially in the treatment of diarrheal disease among children (van Niel et al., 2002; Allen et al., 2004, 2010; Szajewska et al., 2007a; Guandalini, 2008, 2011; Salari et al., 2012). Likewise, there are good data for the treatment of some cases of antibiotic associated diarrhea (D’Souza et al., 2002; Johnston et al., 2006, 2011; Hempel et al., 2012; Videlock and Cremonini, 2012). Especially important for the African scenario with its high infant/child mortality, there is also good evidence from meta-analysis of intervention studies, that probiotics can prevent necrotizing enterocolitis, prevent infant sepsis and decrease mortality in pre-term neonates with low birth weight (Deshpande et al., 2010; Bernardo et al., 2013).

1.2. A global perspective on the use of probiotics

In the Western dietary culture, it is common to ferment milk into yogurt, sour milk or cheese. This long-held custom of fermenting milk explains the high prevalence of probiotic products delivered as fermented milk, particularly sour milk and yogurt in Europe. It is noteworthy, however, that industries in the developed world are creating other delivery vehicles for probiotics, including cheese, whey dairy products, desserts, ice cream, breads, confectionary and soy products as well as meats and fermented vegetables (Gardiner et al., 1998; Ravula and Shah, 1998; Stanton et al., 1998; Hagen and Narvhus, 1999; Lee et al., 1999; Shah and Ravula, 2000; Haynes and Playne, 2002; Ross et al., 2002; Työppönen et al., 2003; Medici et al., 2004; Alamprese et al., 2005; Donkor et al., 2005; Madureira et al., 2005; Kröckel, 2006; Ong et al., 2006; Wang et al., 2006; Alagron-Alegro et al., 2007; Nebesny et al., 2007; Bernardaz et al., 2008; Burns et al., 2008; De Vuyst et al., 2008; Sharp et al., 2008; Cruz et al., 2009; Mäkeläinen et al., 2009; Zollner et al., 2009; De Bellis et al., 2010; Gawkowski and Chikindas, 2013). The world market for ‘functional foods’, i.e. foods which contain ingredients that optimize beneficial properties such as probiotics, prebiotics, vitamins and minerals, had an estimated size in 2003 of ca. 33 billion USD, while the European market estimation exceeded 2 billion USD in the same year. This rose in 2005, to 50 billion USD for functional foods. In western Europe, the consumer market for probiotic foods was > 1.4 billion euros (Saxelin, 2008). Probiotics and prebiotics account for the most important fraction of the overall market for functional foods (Figueira-González et al., 2011), and the world probiotic market share has risen to an estimated 15 billion USD (Bhadoria and Mahapatra, 2011).

In Europe, the largest segments of the functional food markets are represented by foods prepared with probiotics, prebiotics or symbiotics (a combination of probiotics and prebiotics). The probiotic market, especially directed towards yogurts and fermented milks, has experienced rapid growth in the past year. The most developed markets for such products are situated in northern European and Scandinavian countries, where consumers have long tradition of consumption of fermented dairy products (Bhadoria and Mahapatra, 2011). However, due to poor legislative decision making in Europe, some markets have fallen in recent years (Pedretti, 2013). Meanwhile, probiotics have been gaining increasing importance in North America with it being the fourth largest market in the world. More than 100 companies in the USA sell products claiming to be probiotic in supplement form (Sanders, 2008; Bhadoria and Mahapatra, 2011).

Sparse data are available for market share of probiotics in Africa, but it is miniscule in global terms. South Africa appears to have a relatively well-established market with supplements (capsules), fortified food items (especially baby cereals) and fermented dairy products (Brink et al., 2005). The need and interest appear to be there (Anukam et al., 2004; Anukam and Reid, 2005; Reid et al., 2005), in part based upon traditional fermented foods (Anukam and Reid, 2009). However, in line with FAO priorities for food safety in Africa, suggestions have been made for studies on functional (probiotic) properties of LAB strains involved in traditional fermented foods, and to include developing in vitro selection methods for
probiotic strains, followed by studies on technical performance of these strains in traditional substrates (Holzapfel, 2002).

In theory, established probiotic strains and products could be sold in Africa, but a number of critical factors have to be considered. Local populations in developing countries, for example, may prefer a ‘local’ solution to nourishment, health maintenance and restoration. This may reduce the chances that they would purchase a foreign company’s milk product on a regular basis, especially if this product is not part of the normal diet or too expensive in the rural third world setting (Reid et al., 2005). Although the African cultural arena is associated with a great diversity of fermented foods, these are usually based on vegetable protein, cereal or starchy root fermentations and, with some exceptions, less on dairy products, which are dominant in northern Europe and North America. Fermentation of milk in Africa mainly occurs in northern Africa, northern and western Africa, the Sahara and the Savannah and Hills and valleys of East Africa. Such regional products are not typical European type yogurts or cheeses but rather local variations, where various additives such as wood ash, blood or occasionally leafy vegetables may be added and the fermentation vessels are frequently being prepared by smoking (Abdelgadir et al., 1998; Odunfa and Oyewole, 1998; Akinyanju, 1989; Gonfa et al., 2001; Olasupo et al., 2004, 2010; Karenzi et al., 2013).

For Africans, the importance of traditional food fermentation lies in providing improved flavors to existing staples (e.g. cereals and root crops), and as a cheap way of food preservation and an enhancement of the nutritional quality and digestibility of the raw products (Olasupo et al., 2010). Frequently, fermented foods are considered to have health benefits, and in many regions they are believed to aid in the control of some diseases, in particular intestinal disorders (Mathara et al., 2004). Traditional fermented products with improved properties may be more easily accepted by populations with long standing societal structure. An example is an improved ogi, named dogik, that has been developed by Okagbue (1995) using a lactic acid starter with antimicrobial activities against some diarrheagenic bacteria.

Generally, the typical delivery vehicles and flavoring for probiotic foods used in first world countries may not be appropriate for the African situation, especially in a rural setting. Yogurt-type products when not consumed immediately require refrigeration to ensure stability of both the product and the probiotics, but the high ambient temperatures of most regions on the African continent, the great distribution distances and potential problems with maintaining the cold chain in rural areas present logistical problems.

1.3. Why Africa needs probiotics

The reasons why probiotic products containing well-documented strains such as Lactobacillus rhamnosus GG or Lactobacillus casei Shirotia are not yet sold in sub-Saharan African countries are unclear, but may be related either to the problems mentioned before, or because the producing companies do not expect sufficient profit, or suitable distributors may not be available to justify sales (Reid et al., 2005). Sadly, it is in these regions where perhaps the greatest need for probiotics exist (Reid et al., 2005). According to FAO estimates, 925 million people suffered from hunger in 2010, the majority of them living in rural areas. Sub-Saharan Africa is the region of the world with the highest percentage of chronically malnourished people (OECD–FAO, 2011). The reasons for Africa’s serious problem to sufficiently feed itself both quantitatively and qualitatively are numerous, but are based mainly on agronomic constraints and limitations of locally appropriate processing techniques, thus resulting in huge postharvest losses of 30 to 50% (Shiundu and Oniang'o, 2007). It is a tragedy that 14 out of the world’s 20 poorest countries are in Africa, most of which face constantly increasing problems with under- or malnourishment of a major part of the population; moreover, chronic malnutrition has been a persistent problem especially for young children in sub-Saharan Africa. Child mortality in sub-Saharan Africa was reported to be 104 per 1000 live births in 1992, and to range from 51 (South Africa) to 147 (Malawi) per 1000 childbirths in different African countries in 1994 (Kalipeni, 2000). The UN inter-agency group for child mortality estimation reported in 2013 that the highest rates of child mortality are still in sub-Saharan Africa, with an under-five mortality rate of 98 deaths per 1000 live births, this being 15 times the average for developed regions (UN Interagency Group for Child Mortality estimation, 2013). Important to the causal pathway leading to underweight is the malnutrition–infection cycle, whereby underweight children, because of their poor physical stamina and weakened immune system, are at increased risk of infectious diseases such as diarrhea and are thus at substantially increased mortality risk (Mosley and Chen, 1984; Black et al., 2003; Nannan et al., 2007). Feachem and Jamison (1991) estimated that an African child would have a 10% chance of suffering from diarrhea on any given day and a 14% chance of dying from a severe episode. This is significant since diarrhea accounts for 37% of child death deaths in sub-Saharan Africa, being one of the foremost causes of poor health and childhood mortality (Kalipeni, 2000). Thus, Africa is a continent with a high incidence of diarrheal diseases, especially among young children, where child mortality of children less than five years old is extremely high. The utilization of fermented foods containing probiotics would be one avenue by which the health of the children may be improved. Fermentation could also contribute to preserving foods and thus minimizing postharvest losses, as well as detoxifying the raw materials and increasing the intake of macro- and micro-nutrients, thus alleviating malnutrition (Holzapfel, 2002).

Why should fermented foods act as carriers of probiotics, and which strains should be used? As mentioned before, animal protein fermented foods, including milk fermentations, are not widely used in Africa as a whole. While in regions such as Ethiopia and Kenya where milk is fermented and consumed regularly, the production of milk–based products with probiotics would make sense, such products would probably not find wide distribution across the rest of the African continent. Thus, the development of probiotic foods for Africa should ideally rely on traditional fermented foods typical of a region and the local dietary preferences. Or, dried probiotic products should be developed for addition to a variety of local foods, and be able to survive heating in certain cases. Strains used in food fermentations should be well adapted to the product and show good health-promoting activities. In our opinion, this requires the development of new bacterial strains as multifunctional starter cultures or the testing of Western strains in an African setting (Holzapfel, 2002). The development of new probiotics would require investigations into the functional properties of the strain, which might include survival at the body target site, enhancement of the immune response, anti-disease effects, not adversely disrupt the autochthonous microbiota as well as being produced at low cost, be adapted to African food substrates, and retain good viability/survival in the product during storage. Lastly, their effect on properties such as flavor or texture of the food product they will be used in, is certainly also important.

2. An overview of African fermented foods

According to Tamang and Samuel (2010), the world dietary culture has three distinct traditional food habits based on staple cereal diets: (a) cooked–rice eaters of Eastern food culture, (b) wheat/barley-based breads/loaves of Western and Australian food culture, and (c) sorghum/maize porridges of African and South American food culture. Traditional fermented foods still play a major role in the diet of numerous societies worldwide. The African dietary ethos includes both fermented and non-fermented sorghum, maize, millet and cassava products, wild legume seeds and tubers, but also meat, milk products, and alcoholic beverages (Tamang and Samuel, 2010). Of importance to all rural societies are low-cost and, where possible, “low-tech” food processing procedures affordable also by the poor.
In African civilizations, food fermentation still plays a major role in combating food spoilage and foodborne diseases that are prevalent in many of its resource disadvantaged regions. The lactic acid fermentation is probably the oldest and best accepted among the African people (Dirar, 1993), and is largely a home-based process used throughout the continent (Oyewole, 1997). In Table 1, examples of fermentation types and raw materials are given, exemplifying this diversity and expansive nature of the traditions. The legume fermentations, although alkaline and typically dominated by Bacillus spp., are also characterized by the presence of LAB, in particular enterococci, albeit as a minor group. Fermented foods in Africa can be classified in the following major groups based on the raw material used in their production (Olasupo et al., 2010): 1) fermented non-alcoholic cereals, 2) starchy root crops, 3) fermented animal proteins, 4) fermented vegetable proteins, and 5) alcoholic beverages. The first group is predominant and relatively safe, even for infants. Only the fermented non-alcoholic cereals, starchy root crops and animal proteins will be considered below as potential candidates for probiotic foods.

Milk from cattle, sheep and goats is typically fermented in Eastern and Southern Africa, and some regions in North and (more rarely) West Africa, where keeping of such livestock has a long tradition, while camel’s milk is mainly fermented in Northern Africa and the Sudan region. Typical for West African foods are the fermented starchy roots, mainly using cassava (Manihot esculenta Crantz), although cassava fermentation can also be found throughout the continent (Okafor and Ejiofor, 1990; Kimambo et al., 2000; Ray and Sivakumar, 2009). Specific to Ethiopia is the enset (Ensete ventricosum) (also called false banana, Ethiopian banana, or Abyssinian banana) plant, which, can be converted into “kocho” by lactic fermentation (Gashe, 1987), while the (Eragrostis tef), also typical of Ethiopia, is used for preparing the traditional acid-leavened (sourdough) type of “pancake” called injera (Oyewole, 1997; Urga, 1997). Maize (corn), sorghum and millet are traditional acid-leavened (sourdough) type of food fermentations (Oyewole, 1997). Of note, while all these processes involve fermentation, little is known about the range of bacterial strains used to perform the process. This represents an untapped source for future probiotic products.

2.1. Non-alcoholic cereal and vegetable fermentations

Cereals, mostly processed by natural fermentation, account for up to 80% of total calorie consumption in many African countries and they are also an important source of dietary protein. They are frequently complementary foods for young children and as dietary staples for adults. Lactic fermented cereal-based foods in Africa can be classified on the basis of either the raw cereal ingredients used for preparation, or the texture of the fermented product (Nout, 2009; Oyewole, 1997). These include a) maize-based foods such as ogi (Nigeria/West Africa), kenkey (Ghana) and mawe (Benin); b) millet-based foods such as kunuzuki (Northern Nigeria), mbege (Tanzania) and ben-saalg (Burkina Faso); c) sorghum based foods such as ogi-baba (West Africa), bogobe (Botswana), humulur (Sudan) and hussuwa (Sudan); and d) wheat-based foods such as bouza, kishk or kishí (Egypt). Recent studies on fermentation of leafy vegetables (Kasangi et al., 2010) reported that fermentation of cowpea leaves coupled with solar drying could be of potential for small-scale producers as a method of enhancement of nutritive quality. Selected probiotic multifunctional strains could be used to add value to the African indigenous leafy vegetables (Kasangi et al., 2010). In a study by Muchoki et al. (2010), fermentation of Kenyan cowpea leaves followed by solar drying produced a product with reduced anti-nutrients and an increased acceptability, thus potentially alleviating micronutrient malnutrition.

2.1.1. Examples of African fermented maize products

Mawe is an uncooked fermented maize dough, and is an important ingredient for the preparation of cooked beverages, stiff gels, and steamed cooked bread in Benin (Nout, 2009). It is prepared by ‘natural’ or spontaneous fermentation which may be supported by ‘recycling’ or ‘backslipping’ of microorganisms in support of a relatively stable (predictable) process, dominated by heterofermentative LAB such as Lactobacillus fermentum, Lactobacillus brevis, Lactobacillus curvatus, Lactobacillus buchneri, Weissella confusa and pediococci (Hounhouigan et al., 1993, 1994). Typical of most traditional lactic fermentations, yeasts such as Candida krusei, Candida kefyr, Candido glabrata and Saccharomyces cerevisiae may also play some role in the fermentation (Hounhouigan et al., 1994; Nout, 2009). Other examples of fermented maize products include the Nigerian ogi, the Kenyan uji, and the Ghanaian ofata (for making kenkey), although variations occur in procedures, and because millet and sorghum can also be used as raw fermentation materials for the former two (Oyewole, 1997; Nout, 2009). The acidity of fermented uji was shown to correlate with increased shelf-life and to contribute to hygienic safety (Mbugua and Njenga, 1992). Preparation of kenkey, ogi and fermented uji involves soaking of the grains until soft, wet grinding, and fermentation. Fermentation of ogi and uji differs from that of kenkey by the slurring and removal of coarse particles and bran by filtration or sieving, followed by fermentation of the filtrate (Onyango et al., 2004). The uji or ogi filtrates are allowed to ferment near a fire or at ambient temperature for 1 to 3 days. Uji is diluted with water to 8 ± 10% w/v solids and then boiled and sweetened and consumed while still hot, while ogi is boiled to 10% solids into a porridge. Ogi and uji may serve as complementary foods for infants (Onyekwere et al., 1989; Omemu, 2011). The fermentation of uji during sedimentation is predominated by LAB such as Lactobacillus plantarum, L. fermentum, Lactobacillus cellobiosus and L. buchneri, Pediococcus acidilactici and Pediococcus (Ped.) pentosaceus (Mbugua, 1985). In the fermentation of ogi in Nigeria, Odunfa and Adeyele (1985) showed that L. plantarum was the predominant microorganism associated with the fermentation. Other studies showed that besides L. plantarum, the heterofermentative L. fermentum and L. brevis dominated in ogi prepared in Benin or Nigeria (Nago et al., 1998; Omemu, 2011). Omemu (2011) reported a continuous increase in yeast in the fermentation and predominant species included S. cerevisiae, Rhodotorula graminis, C. krusei, Candida tropicalis, Geotrichum candidum and Geotrichum fermentum. In our studies (Sanni et al., 2013) on Nigerian isolates from jufu and ogi we could show that Pediococcus pentosaceus strains dominated (42.1% of isolates) while L. plantarum and L. fermentum strains were also isolated at a high frequency of 24.6 and 26.3%, respectively.

Kenkey has a shelf-life of several days, and is consumed as “ready-to-eat” meal together with other foods (Nout, 2009). Dominant microorganisms during the fermentation are L. plantarum, L. fermentum, L. brevis, L. reuteri and Ped. pentosaceus (Olsen et al., 1995) and yeasts,
mainly *C. krusei* and *S. cerevisiae* (Jespersen et al., 1994). *Amahewu* or *mahewu* is a sour maize-based fermented gruel or beverage consumed mainly by the indigenous people of South Africa. *Amahewu* is prepared by adding 9 parts of water to 1 part maize meal and boiling. The slurry is then cooled to 40 °C and a small amount of wheat flour is added as source of starter inoculum. The gruel is allowed to ferment for 1 to 3 days in a warm place (Chelule et al., 2010). This product has been industrialized in South Africa (Holzapfel and Taljaard, 2004) and, together with its wide acceptability and the established retail distribution channels, it provides a promising basis for an African probiotic product.

### 2.1.2. Examples of fermented sorghum

Hussuwa is a semi-solid, dough-like food of the Sudan. A liquid paste of sorghum flour and water are mixed in equal volumes, cooked to the stiff ‘acea’d porridge, and a further half volume of sorghum malt added and left to ferment for up to 48 h. The resulting sourdough is cooked on a hot plate until all moisture is expelled (El-Nour et al., 1987). After cooking, the hussuwa is fermented in an earthenware pot buried under the fireplace for up to two months, thereby ensuring a continuous warm temperature throughout the period to promote the mixed lactic and alcoholic fermentation (Yousif et al., 2005), finally resulting in a sweet–sour product (Dirar, 1993). A taxonomic study showed that the majority of the LAB strains typical of hussuwa fermentation were heterofermentative lactobacilli, mainly *L. fermentum* while pediococci also predominated. As mentioned above, ogi or kenkey can also be produced with sorghum.

### 2.1.3. Examples of fermented millet

*Ben-sealga*, a thin porridge is prepared by cooking the fermented sediment of pearl millet (*Pennisetum glaucum*) in water, and is typical of Burkina Faso (Nout, 2009). *L. fermentum*, *L. plantarum* and *Ped. pentosaceus* typically dominate the natural fermentation. Some *L. plantarum* strains are able to degrade starch (Ben Omar et al., 2006; Songré-Ouattara et al., 2008) which can be beneficial as they can positively contribute to increase the energy density of cereal based nutritional components of pearl millet such as phytate were reported by adding 9 parts of water to 1 part maize meal and boiling. The slurry is then cooled to 40 °C and a small amount of wheat flour is added as source of starter inoculum. The gruel is allowed to ferment for 1 to 3 days in a warm place (Chelule et al., 2010). This product has been industrialized in South Africa (Holzapfel and Taljaard, 2004) and, together with its wide acceptability and the established retail distribution channels, it provides a promising basis for an African probiotic product.

### 2.2. Starchy root crop fermentations

Cassava is a major root crop processed by lactic fermentation, providing a wide range of safe products such as *gari*, *fufu* and *lafun* or *kokonte* common throughout West Africa, and *kivunde* and *cingwada* in East Africa (Kimaryo et al., 2000; Padonou et al., 2009). The shelf life of cassava is less than 5 days with deterioration starting within 24 h after harvesting. Lactic fermentation drastically prolongs the shelf life, and, for bitter cassava with high cyanogenic glucoside levels, the toxicological safety. *L. plantarum* typically dominates the fermentation process and is inhibitory to many spoilage molds and bacteria, both in the East African (Tanzanian) (Kimaryo et al., 2000) and West African (Ghanaian) fermentation process (Amoa-Awua et al., 1996).

*gari* is obtained by washing and grating fresh cassava roots, dewatering and fermenting at ambient temperature for up to 72 h. The resulting mash is heated and roasted to evaporate the moisture and the resulting flour is the *gari* product (Onyekwere et al., 1989; Kostinek et al., 2005). In contrast to the solid-state fermentation of *gari*, *fufu* and *lafun* are produced in a submerged fermentation of the cassava roots for 4 days, followed by washing, dewatering, drying and milling to obtain a cassava flour. A study on *lafun* fermentation showed that *L. fermentum*, *L. plantarum* and *W. confusa* dominated the LAB population in the fermentation, while predominant yeasts were *S. cerevisiae, Pichia scutulata, Kluyveromyces marxianus* and *Hansenia guilliermondii* (Padonou et al., 2009). In a study conducted on cassava fermentation to produce gari in Benin, *L. plantarum* was the most abundantly isolated species, followed by *Leuconostoc fallax* and *L. fermentum* (Kostinek et al., 2005).

In controlled studies, selected *L. plantarum* strains showed potential as starter cultures for cassava fermentation in the *kivunde* process (Kimaryo et al., 2000) and for the production of gari (Huch et al., 2008). Strains of *L. plantarum*, *L. fermentum* and *Weissella paramesenteroides* were also found suitable for distribution and application as freeze-dried starter cultures for gari production (Yao et al., 2009). Indeed, lyophilized cultures were used to start *gari* fermentations in a rural pilot plant in Quedo village in Benin run by a women's group of 13 members. The use of the starter cultures resulted in rapid acidification of the mash, ensuring a high safety of the final product, and in a reduction of the fermentation period from 24 to 12 h (Egounley et al., 2007).

Some cereal-based, lactic acid fermented products are consumed without heat treatment after fermentation, for instance, the Tanzanian beverage *togwa*, which is often made from sorghum or maize and is consumed regularly by young children (6–60 mo of age). It has been shown to decrease the occurrence of enteropathogens in the rectum and improve the barrier function of the intestinal mucosa in children aged 6–25 months with acute diarrhea (Kingamkono et al., 1999; Willumsen et al., 1997).

### 2.3. Animal protein fermentations

In Africa, animal protein fermentations are mostly related to milk fermentations, although some fish are also fermented. Keeping of livestock with concomitant milk production has a long tradition in South and East regions, where the utilization of fermented milk products is frequently integrated into the societal culture, comprising an important part of the daily diet. Milk fermentation is mainly by traditional, small-scale ‘sour milk technologies’ in rural areas to convert milk into various products for extending shelf life. *Amasi* is a traditional fermented milk consumed in South Africa and Zimbabwe, and its preparation involves fermentation for several days of raw milk in calabashes made of gourd, or in stone jars (Chelule et al., 2010). The major fermented milk products produced in Ethiopia by small holder farmers using traditional methods are: *Ergo* (fermented sour milk), *ititu* (fermented milk curd), *kibe* (traditional butter), *neterkibe* (*kibe* or traditional cheese), *ayib* (cottage cheese), and *arera* (sour defatted milk) (Gonfa et al., 2001). Relatively little is known about the microbiology and presumed dominating LAB of these fermentations. Two studies of traditionally processed *ititu* showed that it contains high microbial numbers of lactobacilli with *L. casei* and *L. plantarum* as dominant species (Kassaye et al., 1991; Gonfa et al., 2001).

The traditional Maasai fermented milk, *kule naoto*, is an important part of the daily diet of the Maasai community in Kenya and Tanzania. These people rarely consume fruits or grains, while their standard daily diet comprises on average 2–3 L of *kule naoto* per person. *Kule naoto* is produced from unpasteurized whole milk from Zebu cows in the rural areas, with spontaneous fermentation in a calabash occurring over at least 5 days. Kenyans appreciate the product for its excellent taste and aroma. In addition, they ascribe functional benefits to consumption of *kule naoto* because of protection against diarrhoea and constipation (Mathara, 1999). In a study on 300 LAB strains isolated from Kenyan traditional *kule naoto*, the genus *Lactobacillus* was found to dominate the fermentation, followed by *Enterococcus, Lactococcus* and *Leuconostoc*. Interestingly, *L. plantarum* was the dominant species, with numbers ranging from 10^7 to 10^8 cfu/ml, while *L. fermentum*, *L. paracasei* and the *L. acidophilus*‘ group were detected at a level of 10^5 to 10^6 cfu/ml (Mathara et al., 2004). The microbiological safety of *kule naoto* was indicated by the absence (detection level < log 2.0/ml) of *Enterobacteriaceae* in all samples (n = 16 out of 22) with pH < 4.5.
Yeasts were present as a minor population at pH values < 4.5, but were not detectable (≤ 1.0/ml) in samples with pH values ≥ 4.5, where numbers of up to log 8.0 per ml of Enterobacteriaceae were present (Mathara et al., 2004).

3. Choice of multifunctional strains for African probiotics

As noted earlier and shown in Table 2, the predominant LAB associated with traditionally African fermented foods generally include lactobacilli and pediococci. Among the lactobacilli, L. plantarum and L. fermentum strains are particularly predominant in many traditional African fermentations (Mbugua, 1985; Olsen et al., 1995; Hounhouigan et al., 1993; Kostinek et al., 2005; Abriouel et al., 2006; Padonou et al., 2009; Njeru et al., 2010; Yousif et al., 2010; Ogutuyo and Narbad, 2012; Owsu-Kwarteng et al., 2012). The well-documented L. plantarum strain 299 V (Probi AB, Lund, Sweden) is also commercially available (Yoba-for-Life (www.yoba4life.com) is one such model in which genericized L. rhamnosus GG is used to produce healthy yogurt in a community kitchen in Uganda, with expansion ongoing to other regions including Zambia (Kort and Sybesma, 2012).

Table 2
African fermented food products and the microorganisms associated with the fermentation (excluding alcoholic beverages) (adapted and modified from Olasupo et al., 2010).

<table>
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<th>Product</th>
<th>Area of production</th>
<th>Fermentable substrate</th>
<th>Microorganisms reported to be involved in the fermentation</th>
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<tbody>
<tr>
<td>African, non-alcoholic cereal based foods</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mahewu (magou)</td>
<td>South Africa</td>
<td>Maize, sorghum or millet</td>
<td>L. delbrueckii subsp. bulgaricus, L. delbrueckii subsp. delbrueckii, Leuconostoc spp.; heterofermentative lactobacilli</td>
</tr>
<tr>
<td>Ogi</td>
<td>Nigeria, Benin</td>
<td>Maize, sorghum or millet</td>
<td>Ped. pentosaceus, L. fermentum, L. plantarum, yeast (Saccharomyces cerevisiae, Candida krusei)</td>
</tr>
<tr>
<td>Koko and Kenkey</td>
<td>Ghana</td>
<td>Maize, sorghum or millet</td>
<td>W. confusa, L. fermentum, L. salivarius, L. vaccinostercus, L. pantheris, Pediococcus spp. and yeast</td>
</tr>
<tr>
<td>Uji</td>
<td>East Africa</td>
<td>Maize, sorghum or millet</td>
<td>L. plantarum, L. paracasei, L. buchneri, Ped. acidilactici, Ped. pentosaceticus</td>
</tr>
<tr>
<td>Kisa</td>
<td>Sudan</td>
<td>Sorghum</td>
<td>L. fermentum, Ped. acidilactici, Ent. faecium (minor proportions)</td>
</tr>
<tr>
<td>Hassuwa</td>
<td>Sudan</td>
<td>Sorghum</td>
<td>Candida guillermondii</td>
</tr>
<tr>
<td>Injera</td>
<td>Ethiopia</td>
<td>Sorghum</td>
<td>L. fermentum, L. plantarum, L. rhamnosus</td>
</tr>
<tr>
<td>Ting</td>
<td>Botswana, South Africa</td>
<td>Sorghum</td>
<td>LAB</td>
</tr>
<tr>
<td>Obusera</td>
<td>Uganda</td>
<td>Millet</td>
<td>L. lactis, Ped. pentosaceticus, L. plantarum</td>
</tr>
<tr>
<td>Mawe</td>
<td>Benin</td>
<td>Maize</td>
<td>L. fermentum, L. paracasei, L. buchneri, Ped. acidilactici, Ped. pentosaceticus</td>
</tr>
<tr>
<td>Kunu-zaki</td>
<td>Nigeria</td>
<td>Millet, sorghum</td>
<td>L. fermentum, P. pentosaceticus, W. confusa, Ent. faecalis</td>
</tr>
<tr>
<td>Bogobe</td>
<td>Botswana</td>
<td>Sorghum</td>
<td>Unknown</td>
</tr>
<tr>
<td>Dégué</td>
<td>Burkina Faso</td>
<td>Millet</td>
<td>L. gasseri, L. fermentum, L. brevis, L. casei, Enterococcus spp.</td>
</tr>
<tr>
<td>Ben saalga</td>
<td>Burkina Faso</td>
<td>Millet</td>
<td>L. plantarum and other LAB</td>
</tr>
<tr>
<td>African fermented starchy root products</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gari</td>
<td>West Africa</td>
<td>Cassava</td>
<td>L. plantarum, L. fallax, L. fermentum (predominating) W. paramesenteroides, L. brevis, Leuc. pseudomesenteroides (minor proportions), Strep. lactis, Geotrichum candidum, Corynebacterium manihot (also reported)</td>
</tr>
<tr>
<td>Lafun</td>
<td>Nigeria</td>
<td>Cassava</td>
<td>L. fermentum, L. plantarum, W. confusa, yeast (Saccharomyces cerevisiae, Pichia scutulata, Klyveromyces marxianus, Hanseniaspora guilliermondii), and Bacillus spp.</td>
</tr>
<tr>
<td>Fufu</td>
<td>Tanzania</td>
<td>Cassava</td>
<td>Ped. pentosaceticus, L. fermentum, L. plantarum</td>
</tr>
<tr>
<td>Chikangwue</td>
<td>Zaire</td>
<td>Cassava</td>
<td>LAB, yeast</td>
</tr>
<tr>
<td>Cingwada</td>
<td>East and Central Africa</td>
<td>Cassava</td>
<td>Unknown</td>
</tr>
<tr>
<td>Kocho</td>
<td>Ethiopia</td>
<td>Ensete or Abyssinian banana (Ensete ventricosum)</td>
<td>LAB yeast</td>
</tr>
<tr>
<td>Agbelima</td>
<td>Ghana</td>
<td>cassava</td>
<td>L. plantarum, L. brevis, L. fermentum, Leuc. mesenteroides, also Bacillus spp., Candida tropicalis, Geotrichum candidum, Penicillium spp.</td>
</tr>
<tr>
<td>African fermented animal proteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nono (milk curd)</td>
<td>Northern part of West Africa</td>
<td>Milk</td>
<td>LAB</td>
</tr>
<tr>
<td>Maziwalala</td>
<td>East Africa</td>
<td>Milk</td>
<td>“Strep.” (Lact. lactis, Strept. thermophilus)</td>
</tr>
<tr>
<td>Leban (sour milk)</td>
<td>Morocco</td>
<td>Milk</td>
<td>Lactococcus lactis, Lactococcus lactis subsp. cremoris</td>
</tr>
<tr>
<td>Wara</td>
<td>West Africa</td>
<td>Milk</td>
<td>Lactobacillus spp., Lactococcus spp.</td>
</tr>
<tr>
<td>Ergo</td>
<td>Ethiopia</td>
<td>Milk</td>
<td>L. plantarum, L. fermentum, L. paracasei, L. acidophilus, also lactococci, leuconostocs and enterococci</td>
</tr>
<tr>
<td>Kulaeno</td>
<td>Kenya</td>
<td>Milk</td>
<td>Lactobacilli, lactococci, yeast (Debaromyces hansenii, Saccharomyces cerevisiae, Cryptococcus curvatus)</td>
</tr>
</tbody>
</table>

The studies on kule naato described above seem to strongly support the ‘recycling’ concept of beneficial microbes between the human environment and the GI tract. Kule naato is typically produced in close association with the traditional Maasai living and cultural environment. The studies by Mathara et al. (2008a,b) and Vizoso Pinto et al. (2007, 2009) have shown strains of L. casei, L. rhamnosus, L. plantarum and, in particular, the L. acidophilus “group” (including L. johnsonii) all typical of the human GI tract (Holzapfel et al., 2001; Heilig et al., 2002; Vaughan et al., 2005), dominate in the Maasai milk fermentation. Moreover, these studies have confirmed their ability to survive passage of the upper GI tract in high numbers, and their potential for beneficial interaction under simulated conditions typical of the human GIT. The potential of some strains for technical implementation has been confirmed (Patrignani et al., 2006), thereby suggesting the ‘multifunctionality’ of at least some of the LAB strains typically associated with fermented Maasai milk.

The Food and Agriculture Organization and World Health Organization (FAO-WHO, 2002) published a guideline pointing out requirements for products to be classified as probiotics. While some of these need to be revised, the basic ones remain important, namely accurate identification of the strains, verification of safety and non-toxicity and the ability to provide tangible physiological/health benefits as shown in randomized clinical trials. For testing safety, the European Food Safety Authority (EFSA) QPS system for safety assessment may be utilized (EFSA, 2005). The approach for determining safety according to this document is based on a safety decision tree. The EFSA took on the task to develop the QPS system within an EU regulatory framework. For each strain, safety needs to be shown based on the ‘body of knowledge’ of the strain, including description of known virulence determinants, as well as antibiotic resistance patterns, some of which are intrinsic and not generally transferred to other strains.

Studies that relate only to in vitro investigations on adhesion properties, pathogen inhibition and stimulation of immune parameters in cell culture, for example, are not sufficient to call a strain probiotic (FAO/WHO, 2002; Reid, 2005; Anukam and Reid, 2009). Nevertheless, in vitro investigations are good to inform about strain properties. Functional cell models of the gut can be very useful to investigate potential probiotic activities of strains. Simple cell cultures models involving small intestine or colon enterocytes such as Caco2 or HT29 cells have often been used to investigate probiotic activities of LAB strains as relating to adhesion or prevention of pathogen invasion. Improved functional mammalian cell cultures are becoming more and more available, which consist of cells grown on a microporous membrane in a well, and which are underlaid with immune cells such as dendritic cells or macrophages in so-called 3-D or trans–well cell cultures (Cencic and Langerholc, 2010). As these consist of three components (epithelia, immune cells and microbiota) the models are closer to the in vivo situation and are thus better suited for studying probiotic activities such as adhesion or host/beneficial bacteria/pathogen interaction (Cencic and Langerholc, 2010), as well as for assessing aspects of strain safety. Validated, in vitro gastrointestinal models such as the TIM–1/TIM–2 or the (M)SHIME models, are also excellent tools to study probiotic activities such as potential survival in the gastrointestinal tract and their effect on the gut microbiota homeostasis after antibiotic treatment (Rehman et al., 2012; Venema and van den Abbeele, 2013).

For a product to be classified as probiotic, however, it needs to have been proven to confer a health benefit. For this, well-controlled human clinical trials are required (Anukam and Reid, 2009). In the age of metagenomics, these trials should ideally also include sequencing of the appropriate microbiome (gut, oral, skin, vagina) and determination of the impact of the probiotic on microbiota homeostasis. The human gut microbiota has received considerable attention in recent years and metagenomic studies have shown a large variability in the microbiota composition of individuals (Arumugam et al., 2011). Although sizeable variation between individuals exists, most can be categorized as belonging to one of three groups, so-called ‘enterotypes’, based on the predominance of either of the genera Bacteroides, Prevotella or Ruminococcus (Arumugam et al., 2011). However, these groups may be better characterized as the ratio of abundance of Bacteroides and Prevotella, with the Ruminococcus enterotype folded into the Bacteroides group (Wu et al., 2011; Clemente et al., 2012). Host genetics seem to play a role in establishment and shaping of the gut microbiota (Benson et al., 2010; Spor et al., 2011), but diet is the driving force for the formation of the different enterotypes (Wu et al., 2011).

So far only limited studies have focused on the gut microbiota of African individuals, and usually not in the context of probiotic intervention trials. In one study, De Filippo et al. (2010) used a metagenomic approach to compare the composition of the gut microbiota of children (1–6 y) from Italy, who consume a Western-style diet (high in starch, sugar and animal protein, low in fiber), and from rural Africa (Burkina Faso), whose diet consists mainly of cereals, legumes and vegetables (high in fiber, carbohydrates and non-animal protein). The gut microbiota of the African children showed a highly significant enrichment in Bacteroidetes and a depletion in Firmicutes, with a unique abundance of bacteria from the genera Prevotella and Xylanibacter which contain genes for cellulose and xylan hydrolysis, that were completely lacking in the EU children. Furthermore, the gut microbiota was far more complex than that of EU children. It has been suggested that the gut microbial richness may have a health promoting effect by offering better protection against pathogens and gastrointestinal disease. The authors hypothesized that the African diet correlated with a gut microbiota that allowed maximum energy intake from fibers while at the same time protecting against inflammation and non-infectious colonic disease (De Filippo et al., 2010).

A later comparative study on the gut microbiota of 6 month old infants from Malawi and Finland showed considerable differences (Grzeskowiak et al., 2012) with that of the former in that the gut microbiota of Malawian children was dominated by bifidobacteria and the proportion of Bacteroides–Prevotella group was also much higher. On the other hand, Bifidobacterium adolescentis, Clostridium perfringens and Staphylococcus aureus were absent in Malawian, but detected in Finnish infants. The authors noted that B. adolescentis has been associated with inflammatory effects and that allergic infants are often colonized by C. difficile and S. aureus, but less so by bifidobacteria (Collado et al., 2010; Grzeskowiak et al., 2012). Interestingly, higher levels of Clostridium species and staphylococci have been reported in overweight infants and adults (Ley et al., 2005; Collado et al., 2010; Grzeskowiak et al., 2012).

One study investigated the fecal microbiota of African children with health problems, and showed that anemic children from the Ivory Coast carry an unfavorable high ratio of fecal enterobacteria to bifidobacteria and lactobacilli, which was even increased by iron fortification (Zimmermann et al., 2010). A further study showed that the gut microbiota was a causal factor in the development of kwashiorkor, an enigmatic form of severe acute malnutrition in Malawian children that appeared to be characterized by Bilophila wadsworthia, a bacterium linked to inflammatory bowel disease, and Clostridium innocuum, a species which can function as an opportunist in immunocompromised hosts (Smith et al., 2013).

From the above studies (De Filippo et al., 2010; Zimmermann et al., 2010; Grzeskowiak et al., 2012) it appears that in healthy African children a diet high in fiber and carbohydrates appears to select a microbiota that is highly diverse, protective against infection and inflammation and which extracts maximum energy from the low calorie food. This supports the potential use of indigenous fermented plant foods as vehicles for probiotics in the African setting. In terms of disease such as anemia or severe under nutrition in children, fermented foods may become more important to affect the gut microbiota. Restoration of gut functionality by interventions that stimulate the diversity of bacteria, and through probiotics with immune stimulatory activity, appears to hold great promise and should be further investigated.
A number of studies on the in vitro probiotic potential of predominant LAB isolated from African fermented foods have been carried out, as will be discussed below. However, their potential health benefits have unfortunately not been subject to many intensive investigations in well-controlled, randomized clinical trials in Africa. To date, no metagenomic studies have accompanied such intervention studies. The severe lack of government investment in R&D and lack of industry and philanthropic funding makes this a major challenge. For selecting probiotic foods in the African setting, both the probiotic strain(s) and the food matrix need to be considered.

3.1. The search for potential probiotic strains to supplement African fermented foods

Table 3 gives an overview of strains studied as potential probiotic candidates for use in African fermented foods. Banwo et al. (2013) investigated Enterococcus faecium strains isolated from cow’s milk for their technological properties and probiotic potential in a fermented milk product nono. Two strains were shown to be tolerant to bile salts, possess antagonistic activity against foodborne pathogens including Bacillus cereus and Listeria monocytogenes and showed strong acidification properties. The strains were non-hemolytic and exhibited no antibacterial resistance. Although there are some well-known examples of probiotic enterococci on European markets, these bacteria are still often viewed as problematic because many strains belonging to specific genetic subsets are known to be involved in nosocomial infections (Franz et al., 2011b).

Jacobsen et al. (1999) investigated L. plantarum and L. fermentum maize dough strains from Ghana and found that some were poorly adherent to Caco-2 cells but survived 4 h at pH 2.5, while growth was not delayed in 0.3% oxgall. Some strains showed antimicrobial activity towards L. monocytogenes, S. aureus and B. cereus strains, but products have not been tested in humans.

In an investigation of potential technological/probiotic characteristics of LAB from fufu and ogi, Sanni et al. (2013) showed that many strains, especially pediococci, produced H2O2, and were tolerant towards Enterococcus faecium. Banwo et al. (2013) showed resistance to gastric juice and bile, while some expressed bile salt tolerance. Genetic screening revealed a potential for folate and riboflavin synthesis in both the metagenomes and the isolates. The idea is for LAB capable of producing B vitamins to be used to fortify cereal based foods (Turpin et al., 2011). A study by Njeru et al. (2010) showed that L. fermentum was also the dominant species isolated in Kimere, a spontaneously fermented pearl millet dough produced in Kenya. All the L. fermentum strains were able to grow in vitro in medium containing 0.3% oxgall and 12 strains even grew in the presence of 3% oxgall. Of the latter, 60% of the strains survived incubation at pH 3 for 3 h.

Our studies on LAB from the Maasai fermented milk product kule naoto (Mathara et al., 2008a,b) aimed to investigate the functional characteristics of the predominant bacteria. Lactobacillus paracasei, L. plantarum, L. rhamnosus and isolates of the L. acidophilus group showed resistance to gastric juice and bile, while some expressed bile

<table>
<thead>
<tr>
<th>Region/country in which the fermented African food product is commonly consumed</th>
<th>Strains</th>
<th>Source of strains</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigeria, Sub-Saharan Africa</td>
<td>Raw cow milk for the production of fermented milk product nono</td>
<td>Enterococcus faecium</td>
<td>Banwo et al. (2013)</td>
</tr>
<tr>
<td>Ghana</td>
<td>Fermented maize dough, Cassava</td>
<td>L. plantarum, L. fermentum</td>
<td>Sanni et al. (2013)</td>
</tr>
<tr>
<td>Ghana</td>
<td>Fermented millet flour</td>
<td>L. fermentum, L. plantarum</td>
<td>Jacobsen et al. (1999)</td>
</tr>
<tr>
<td>Ghana</td>
<td>Fermented millet dough, Papaya, Pumpkin</td>
<td>L. fermentum</td>
<td>Jacobsen et al. (1999)</td>
</tr>
<tr>
<td>Uganda, Kenya</td>
<td>Fermented milk product</td>
<td>L. johnsonii, L. paracasei, L. acidophilus, L. fermentum</td>
<td>Cho et al. (2010), Nielsen et al. (2010), BFE 5092, BFE 5759, BFE 5878</td>
</tr>
<tr>
<td>Nigeria</td>
<td>Milk yogurt and milk yogurt with Moringa powder</td>
<td>L. paracasei, L. pentosus, L. rhamnosus, L. acidophilus, L. casei, L. plantarum</td>
<td>Wenner (2009), Van Tienen et al. (2011)</td>
</tr>
</tbody>
</table>

Table 3 Overview of strains studied as potential candidate probiotic strains for use in different African fermented food.
salt hydrolase activity and the ability to assimilate cholesterol in vitro. Adhesion to HT29 MTX cells of up to 70% of total bacterial cells added to the HT29 MTX cells was recorded after 1 h incubation, and also a high survival rate under simulated stomach acidic conditions and physiological bile salt concentrations (Mathara et al., 2008a,b). The choice of cells used for adhesion studies in cell cultures is quite critical to determine the adhesion capability of potential probiotic strains. Caco2 and HT29 cells do not produce mucus, while the HT29-MTX cell line is a mucus-producing cell line which mimics the intestinal situation more closely. Thus, binding assays can obtain quite different results when using HT-29 and HT29-MTX cell lines, as reported by Turpin et al. (2012), who showed that some, but not all, strains from fermented foods have higher binding capability on HT29-MTX cells when compared to HT29 cells. Quite interesting, in that study, some of the strains from African fermented pearl millet showed a far higher binding capability to HT29 and HT29-MTX cells than established probiotic strains that are on the market (Turpin et al., 2012).

In our studies (Mathara et al., 2008a,b) technological features such as growth kinetics in fresh heat-treated whole milk medium and survival in the final product during storage at 4 °C were also studied for several strains and L. acidophilus BFE 6059, L. paracasei BFE 5264 and Lactococcus lactis BFE 6049 from kule naoto showed potential for use as starters for milk fermentation (Patrignani et al., 2006). Five strains identified as L. plantarum and two as L. johnsonii showed good survival under simulated gastrointestinal conditions, and expressed antimicrobial activity. All strains exhibited bile salt hydrolase activity, while, interestingly, only the L. plantarum strains showed ß-galactosidase activity (Vizoso Pinto et al., 2006). The strain BFE 6128 was identified as L. johnsonii, and confirmed to have interesting potentially probiotic properties (Vizoso Pinto et al., 2007, 2009). It was also studied for modulation of signal pathways involved in innate immunity in enterocytes. It sensitized HT29 intestinal epithelial cells towards recognition of Salmonella enterica serovar Typhimurium by increasing the IL-8 levels released after challenge with this pathogen, and by differentially modulating genes related to toll-like receptor (TLR) pathways and innate immunity.

Other L. plantarum strains from kule naoto showed potential as probiotics as they adhered well to enterocytes and prevented invasion of pathogens in cell culture. They expressed antimicrobial activity towards foodborne pathogens and stimulated IL-8 production in intestinal epithelial cells in vitro (Vizoso Pinto et al., 2009). One of these strains, L. plantarum BFE 5092, had a bacteriocin locus and the genes for production of plantaricins EF, JK and N (Cho et al., 2010), with antimicrobial activity towards L. monocytogenes (Nielsen et al., 2010). In summary, the pool of potential probiotic strains is sizeable, at least based upon features believed to be useful in the gastrointestinal tract.

3.2. Intervention studies with African potential probiotics

Animal experiments have provided some rationale for human studies in Africa. Opere et al. (2003) showed Lactobacillus starter cultures of a fermented cereal gruel prevented shigellosis in a murine animal model. Whereas all control mice died upon Shigella dysenteriae infection, 80% of mice and 100% of mice previously receiving a diet of an African fermented complementary food for young children containing L. acidophilus and L. pentosus survived, depending on the starter strain or combination of strains used to ferment the gruel. Lei and Jakobsen (2004) studied the microbiology of the millet porridge koko and koko sour water (the fermented liquid top layer developing during koko fermentation) produced in Ghana containing approx. 10^8 live LAB per ml and proposed it as a probiotic drink. An intervention study was undertaken in which children of less than 5 years of age coming to northern Ghana health clinics for treatment of diarrhea were randomized, to receive treatment for diarrhea and in addition up to 300 ml fermented koko sour water daily or a control drink for 5 days after enrolment (Lei et al., 2006). Among the 184 children included, no effects of the intervention were noted for stool frequency, stool consistency and duration of diarrhea, however, the koko sour water treatment was associated with greater reported well-being 14 days after the start of the intervention. Many of the children received antibiotics as normal part of the treatment, and this was suggested to have masked probiotic effects.

With an initial goal of alleviating gastrointestinal abnormalities among HIV infected subjects in sub-Saharan Africa, a community kitchen project was established in Mwanza, Tanzania in 2004. Local mothers were taught to produce yogurt supplemented with probiotic L. rhamnosus GR-1 (named Fiti), with good sensory and anti-diarrheal properties (Hekmat and Reid, 2006; Anukam et al., 2008). This grass roots initiative holds great potential to deliver health and nutritional benefits to a wide population (Wenner, 2009; Reid, 2010). It was hoped the yogurt could improve gastrointestinal integrity and reduce the incidence and severity of opportunistic infections among people with HIV. Irvine et al. (2011) performed a retrospective study, and preliminarily results suggested that yogurt supplemented with L. rhamnosus may effectively alleviate GI symptoms and improve workforce productivity, nutritional intake and tolerance to antiretroviral treatment. Further support for these benefits has emerged (Reid, 2010; Hummelen et al., 2011a; Whaling et al., 2011), but more studies are needed. The addition of micronutrients to the probiotic Fiti was designed to further improve gut health and nutrient status, and two different formulations offer hope in that regard, especially locally sourced Moringa (Hemsworth et al., 2011, 2012; Hummelen et al., 2011b; Van Tienen et al., 2011).

4. Further perspectives

Despite arguably Africa having the greatest need for probiotics with anti-infective characteristics, the introduction of products remains a forlorn hope more than a reality. The perception that the marketplace is too small is short-sighted, and while cold chain networks and effective transportation systems are believed to be poor, they do not prevent major soft drink producers from establishing vast product distribution across the continent. Indeed, if sugar-laden drinks can be affordable to large populations, often being cheaper than bottled water, why can’t probiotic drinks be made available? Companies need to ask these questions themselves, and recognize that the African market potential is extremely large in the long term.

There is undoubtedly disparity of income in many African countries, like everywhere else, but the extreme poverty, malnutrition and exposure to malaria, tuberculosis and HIV make the plight of the poor all the more catastrophic. Failure to transfer probiotic products to Africa that are currently improving the lives of hundreds of millions of people elsewhere, is tantamount to a neglect. Nevertheless, for those scientists and businesses trying to make a difference, solutions are slowly being explored. These include: (i) the establishment of networks in which local people can set up their own kitchens and produce probiotic foods; (ii) the isolation of African bacterial strains from traditional foods, and developing them for probiotic applications; and (iii) attempts to industrialize fermented food processing to account for regional variations in raw materials, recipes and production methods (Nout, 2009), and starter culture technology (Holzapfel, 1997, 2002). In terms of government engagement, the situation remains dire even as countries emerge from extreme poverty and coping with the HIV crisis. Infrastructure development in South Africa, Zambia, Rwanda, Kenya and other countries is clear for all to see, and investment especially from China, as well as philanthropic contributions from non-governmental organizations and the Bill and Melinda Gates Foundation and Welcome Trust, to name two, have made significant differences. Yet, governments are not investing in funding their own scientists to develop novel foods and health remedies. This needs to change. A roadmap for R&D in probiotics was proposed 8 years ago, without success: a) funding of trials at the local level to assess the applicability to different populations,
Furthermore, new products can be created, such as introduced to other fermented slurries or gruels such as Togwa before heating (as in the case of hussuwa by steeping and malting sorghum grains, followed by heating, drying, bacteria, a portion of the dant micronutrients, if suitable cooking and storage conditions can be of the rich variety of indigenous vegetables, many of which are abundant in distribution throughout Africa should be a priority and their consumption is crucial for controlled fermentations from household to small to medium enterprise scale.

5.2. Choice of product for probiotic delivery

In our view, cereal or starch/cereal mixed fermentations, with a wide distribution throughout Africa should be a priority and their consumption should be emphasized. To circumvent heat killing of the beneficial bacteria, a portion of the finished fermented product could be ingested before heating (as in the case of koko sour water), or products promoted that do not require heating, like the South African maize grit amaluwe the Sudanese hussuwa or the Tanzanian cereal root crop beverage Togwa which is cooked before fermentation. It is worth investigating whether a portion of the fermented material could be re-introduced to other fermented slurries or gruels such as ogi or uji. Furthermore, new products can be created, such as ‘sorghurt’ produced by steeping and malting sorghum grains, followed by heating, drying, mixing with water, inoculation with starter culture and fermentation (Sanni et al., 2013).

Products that use traditional milk fermentation similar to amasi, ergo or kule naoto can be produced immediately if there is a will in regions of Africa where milk is plentiful. Different products could take advantage of the rich variety of indigenous vegetables, many of which are abundant micronutrients, if suitable cooking and storage conditions can be resolved (Kimijwe et al., 2007). It is time to take advantage of highly nutritious plants that convey health benefits. In addition to Moringa, there is amaranth (Amaranthus spp.), African nightshades (Solanum spp.), spiderplant (Gynandropsis gynandra) and cow pea leaves (Vigna unguiculata). Results from the limited metagenomic studies on the gut microbiota of African children indicate a richness of microbeota in healthy African children with a predominance of Bacteroides associated with the high fiber, non-animal protein and carbohydrate rich diet. The fermentation of indigenous leafy vegetables with multifunctional starters would be especially interesting for further development. While there is always a need for more research, there is already substantial evidence for using fermented foods to impact the health of Africans, especially given the levels of disease and malnutrition.

5.3. Improving research infrastructure

There is certainly cause for optimism, with the presence of research centers in South Africa, Kenya and Nigeria with expertise in microbiome research, and many sites including government run Ministries of Agriculture, where small strides are being made to merge local foods with health benefits. The establishments of probiotic and/or Nutraceutical Centers of Excellence are achievable, whether based at local universities, hospitals, research institutions or independent sites, and would give African probiotic research a needed boost. The problems of malnutrition and disease, child mortality, poverty and social inequity can only be solved by collective and collaborative ventures whose pre-eminent focus is the betterment of fellow humans. As the Scottish Early Years Collaborative is demonstrating, it is through broad societal partnerships that change can occur. The establishment and funding of further close scientific collaborations between Europe and Africa should be a priority to address the problems of malnutrition, disease and child mortality. It might seem strange or even contradictory to some that microbes and fermented foods harbor such potential for paradigm shifting change. But, after all, humans are mostly microbial in content, food is their necessity and as multiple recent studies are showing, these organisms are integral to all aspects of human and animal development, longevity and ultimate survival (de Vos and de Vos, 2012; Hughes, 2012; Trivedi, 2012; Biagi et al., 2013; de Vos and Nieuwdorp, 2013; Fang and Evans, 2013; Llosalco, 2013; Pennisi, 2013; Reid et al., 2013b). We wonder who is willing to join this friendly revolution.

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