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# Development of Probiotics as Biotechnology - Driven Product for Reducing the Incidence of Gastrointestinal Related Disease

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**Abstract:** ***Advances in biotechnology have made a significant progress in development of science driven products leading to reductions in disease risks. Probiotics is one of these products and is seen as live micro-organisms which, when administered in adequate amounts, confer health benefits on the host. Research over the past years have revealed that gut health is critical to overall wellbeing of an individual and that unhealthy guts contributes to a wide range of health challenges observed in nature. Therefore better understanding of gut microbes and their role in health benefit will lead to more products development and less disease incidence. This article present proof of concepts of dietary intervention with probiotics as designed to modulate the gut microbiota for health advantage, progress in the development of probiotics; probiotics research and mechanism, proliferation of commercial probiotics are discussed among others.***

**Keywords:** Probiotics, gut flora, *Bifidobacteria, Lactobacillus acidophilus,* Gastrointestinal tract.

## 1. Introduction

The history of probiotics can be traced back to the era when yoghurt, cheese and other fermented products were first developed and well known to the early Romans empire, Creeks and Egyptians who recommended their consumption (Gismondo et al ., 1999). The fermentation of dairy foods represents one of the oldest techniques for food preservation (Azizpour et al., 2009). Today, Probiotics have received global attention from researchers, product manufacturers, and consumers. The community of bacteria living in the human guts consists of about 100 M microbes which influence health and play a role in the development of diseases. At least different species of these bacteria lives in the gastrointestinal tracts. Whereas one third of this gut microbes are popular among some people, two third are specific to each individual, effectively giving a fingerprint of intestinal wellbeing (Sean, 2015).

The original proof of concepts of the positive role played by certain bacteria was first shown in 1907 by Russian scientist Élie Metchnikoff - a Nobel laureate. He suggested that it would be possible to modulate the gut flora, principally to replace harmful microbes with useful ones (Metchnikoff, 1907). In his study, he indicated that aging process results from the activity of proteolytic bacteria producing toxic substances in the large bowel. Proteolytic bacteria such as clostridia, are part of the normal commensal gut flora, but produces toxic substances including phenols, indols and ammonia from proteins hydrolysis and these compounds were responsible for the physiological changes associated with old age. Metchnikoff had also observed that certain rural populations in Europe, specifically Bulgarian and the Russian who lived largely on milk products, fermented by lactic-acid bacteria were exceptionally long lived.

However, it is known that milk fermented with lactic-acid bacteria inhibits the growth of proteolytic bacteria because of the low pH produced by the fermentation of lactose. The consumption of fermented milk would "seed" the intestine with non-virulence lactic-acid bacteria and reduce the intestinal pH and thus colonized and suppress the growth of proteolytic bacteria. *Bifidobacteria* were the first isolated non-virulence microbes from a breast-fed infant by Henry Tissier. The isolated bacterium named Bacillus bifidus communis (Tissier, 1900), was later renamed to the genus *Bifidobacterium*. They are dominant in the gut flora of breast-fed babies and have observed clinical benefits for treating diarrhea in infants. The claimed mode of action of bifidobacterium was the displacement of proteolytic bacteria which cause gastrointestinal related disease. With the outbreak of shigellosis in 1917 at a time when antibiotics were not yet discovered, methods of treating infectious diseases were highly needed. A certain strain of *Escherichia coli* (*Escherichia coli* Nissle 1917) from the feces of a soldier who was not affected by the disease was used in acute gastrointestinal infectious salmonellosis and shigellosis. Later in 1935 certain strains of *Lactobacillus acidophilus* were found to be very active when taken into the digestive tract especially to relief chronic constipation (Rettger et al., 1935).

The term "probiotics" was first used by Werner Kollath in 1953 to describe food supplements applied to restore health to patients suffering from malnutrition (Hamilton-Miller, 2003). Probiotics was defined as microbial derivatives that stimulate the growth of other microorganisms. The concept of probiotics was used by Lilly and Stilwell (1965), to refer to microorganisms that impact and colonized other microorganisms. Their concepts of probiotics connote the notion of substances secreted by one microorganism to stimulate the growth of another microorganism. Sperti (1971) was in agreement when he describes tissue extracts of bacteria which stimulate microbial growth. In the same line, Parker (1974) defined probiotics as, “organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance”. Later, this definition was greatly improved by Fuller (1989) as a "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". Two important facts of probiotics were stressed: the viable nature of probiotics and its capacity to help with intestinal

balance. However, „probiotic" is a Greek word meaning „for life", and is the opposite of the name antibiotic (Sharifuzzaman, 2010).

In the new millennium, current biological processes for the treatment of gastrointestinal related diseases have undergone considerable evolution and one of the major achievement beside the discovery of antibiotics is the development of probiotics which is described as science-driven product, developed using intestinal lactic acid bacterial species with acclaimed health benefit including *Lactobacillus rhamnosus*, *Lactobacillus casei*, and *Lactobacillus johnsonii* (Tannock, 2003). It has found applications in life sciences, in human healthcare, animal health, aquaculture, poultry, piggery etc.

## 2. Development of novel probiotics

Different source of potential probiotics is currently in used (Verschuere *et al.* 2000; Hong *et al.* 2005). And they include bacteria (Verschuere *et al.* 2000; Gram and Ringø 2005), bacteriophages (Nakai and Park 2002), yeasts (Tovar *et al.* 2002) and microalgae (Austin *et al.* 1992).

A search approach to isolate candidate probionts could be made among the intestinal microbiota of healthy individuals on the assumption that the gut is the natural location of beneficial micro-organisms (Gullian *et al.* 2004). These putative micro-organisms should be (i) non-virulent and non-pathogenic to the host, (ii) have the ability to colonized and inhibit the growth of other intestinal pathogens, (iii) adherence to gut walls and grow within the host, and (iv) be indigenous or adaptable to the intestinal environment to which they will be subjected to (Gatesoupe 1999; Verschuere *et al.* 2000). Research studies have demonstrated that naturally occurring bacteria, i.e. the indigenous microbiota of sprouted grains, fermented soy milk, breast fed babies, healthy adults, fish or animals may produce a range of antagonistic compounds that can inhibit the growth of virulent pathogens (Robertson *et al.* 2000, Spanggaard *et al.* 2001, Vine *et al.* 2004a; Fjellheim *et al.* 2007). Such organisms may be invaluable as probiotics (Hai *et al.* 2009).

In the process several inhibitory assays have been used to develop putative probiotics, such as the cross-streak method, double-layered method, spot-on-lawn method, disc-diffusion, well-diffusion and co-culture methods (Brunt and Austin 2005; Hai *et al.* 2007). The cross-streaking method is popularly in use and allows a comparison of inhibitory activity for different putative probionts (Hai *et al.* 2007). This process may involve experimental examination of possible harmful effects of the selected bacterial isolates on animal host. This could be achievable by intramuscular/intraperitoneal injection. Discovery of non-virulent or non-pathogenic outcome will end further evaluation of the putative probionts *in vivo* against selected pathogen choosing a delivery method. Finally, the beneficial strain is identified as probiotic (Brunt and Austin 2005).

## 3. Probiotics mechanism and Research

Probiotics are very effective in facilitating the digestive progress through multiple mechanisms including digestive enzymes production, immune modulation, gut microbial colonization and improvement in gut environment where they exert anti-carcinogenic, antidiarrhoeal and hypocholestero-laemic effects (Sanders, 1999; Steer *et al.*, 2000). However, the relationship between probiotic bacteria and their hosts is very complex, and the possible modes of actions of probiotics are not completely understood. However, the effect on reducing gastrointestinal related diseases may be linked to a combination of factors listed below:

1) Host immunostimulation, i.e. enhancement of humoral and cellular immune response (Panigrahi *et al.* 2005; Salinas *et al.* 2005; Diaz-Rosales *et al.* 2006),
2) Inhibition of potential pathogens or competition for adhesion sites for nutrient/energy sources) (; Brunt *et al.* 2007; Decamp *et al.* 2008),
3) Serves as source of nutrients or enzymatic contribution to digestion and thus enhanced feed efficiency (Tinh *et al.* 2008; Sáenz de Rodrigáñez *et al.* 2009),
4) Serves to improve water quality and improvement in the intestinal microbial balance (Lallo *et al.* 2007; Hill *et al.* 2009; Picchietti *et al.* 2009).

Probiotic strains have been confirmed through their genome sequencing to contain no plasmids, antibiotic resistant genes or deleterious genes. However, human clinical study has also showed their ability to control microbial populations, causing improvement in digestion and maintenance of general health (Robinson and Samona, 1992).

Feed supplemented with probiotics can serves as therapeutic for gut health improvement. Therefore, it is very appropriate for use after a couple or a course of antibiotics treatment to restore the homeostasis of gut cells. Besides food consumption being the conventional way of supplying the body with nutrients, this functional food can provide the best way as they especially convey life nutrients to the host. Different strains of probiotics have been known to offer specific benefits, this necessitate the increasing consumers populations needs for a multi-strain formulation of both non-spores and spores forming probiotics such as *bacillus subtilis* which support digestive health and work complementary to many non-spore strains. In general, commercial cultures of probiotic bacteria are species of *Lactobacillus* and *Bifidobacterium* that inhabit the human intestine and impart through their presence, unique and beneficial effects on the health of the individual (Holzapfel *et al.*, 1998).

Probiotics research starts with the selection of a strain of bacteria or yeast that has been characterized and found to be non virulence and having health benefits. There are many in vitro microbiological, biochemical, and immunological techniques [Walker and Buckley, 2006] available that may be used in appropriate combination to identify and characterize the selected strains. Here diligence is required in their preparation in an environment where other micro-organisms are present without safeguards to prevent or detect inadvertent contamination.

## 4. Proliferation of Commercial Probiotics

Substantive evidence of health benefits exist for probiotic bacteria and benefits are specific to individual strain. In most

countries of the world, a strain intended to be developed and marketed as a probiotic bacteria for prevention of a specific disease condition is regarded as a live therapeutic product or biotherapeutics and are regulated as a biological drug (Vaillancourt, 2006). Example is the regulation of biological products by the US Food and Drug Administration (FDA) and in Nigeria, the National Agency for food and drug administration and control (NAFDAC). In recent years, we have witness consumers being too health conscious and increasingly looking for products with improved taste, lifestyle-friendliness and functional food. This is also seen in some quarters as a tip of the iceberg when it comes to innovative application in products development. Probiotic manufactured products comes in different forms and packages: probiotic fortified food, probiotic beverages for children, probiotics for athletes, for active seniors, probiotic yoghurt, probiotic soy milk etc have all gain popularity worldwide. Their popularity have caused more consumers lost interest in taking pills, they rather want to receive the full benefit of probiotics without adjusting their daily routine. Organic foods are always accepted in Nigerian context than any chemotherapeutical pills, hence probiotic organisms are produced on a large scale and the cells are dried, formulated and prepared as fortified foods, beverages, yoghurt, dairy products and most often as capsule etc that are taken as dietary supplements to be reestablish in the host guts, thereby gaining back the normal enteric flora and improving the microbial balance.

The fundamental question is how dietary probiotics may induce disease resistance in humans? The answer is not conclusive since an array of possible modes of action have been suggested, which might have complex interrelationships among themselves. It is apparent that the potential effects of probiotics include competitive exclusion (i.e. production of inhibitory compounds, competition for chemicals, oxygen or available energy, and competition for adhesion sites), inhibition of virulence gene expression or disruption of quorum sensing molecules, immunostimulation, a source of macro and/or micronutrients, improvement in the microbial balance, and enzymatic contribution to digestion. Likewise, it has been stated that this group of bacteria should be non-pathogenic, acid resistant i.e. the capacity to survival during transit through the stomach and be persistence in the intestinal tract; bile tolerant and production of antimicrobial substances, including, organic acids and hydrogen peroxide and bacteriocins (biologically active proteins), principally gastrointestinal viability is highly desire in order to provide beneficial effects for the host (Dunne et al., 1999).

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