III.A.7.2

ARTICLE

PROBIOTICS AND GUT HEALTH

Dr.Sunarjati Sudigdoadi,dr.,MS.,SpMK (K)
NIP.1951 1202 198001 2001

FACULTY OF MEDICINE PADJADJARAN UNIVERSITY
BANDUNG
2015

PROBIOTICS AND GUT HEALTH (*)

[redacted]

Department of Microbiology and Parasitology

Faculty of Medicine, Universitas Padjadjaran

Abstract

Probiotics are live microorganisms that are able to reach the gastointestinal tract and alter its microfloral composition, producing beneficial health effects when consumed in adequate amounts. Recent clinical trials have evaluated the clinical effectiveness of probiotics in the treatment and prevention of wide range of acute and chronic gastrointestinal diseases, and also non gastrointestinal diseases. The gastointestinal microbiota plays an important role in host health due to its involvement in nutritional, immunologic and physiological functions. Microbial imbalances have been associated with enhanced risk of spesific diseases. The mechanism of action include the inhibition of pathogen growth by competition for nutritional sources and adhesion sites, secretion of antimicrobial substances, toxin inactivation. The well-characterized immunomodulatory potential of specific probiotics strains, beyond the effect on the composition of the microbiota, has been be used as innovative tools to alleviate intestinal inflammation, normalize gut mucosal dysfunction, and down regulate hypersensitivity reactions. Clinical efficacy of spesific probiotics strains has been demonstrated in, rotavirus's diarrhea, antibiotic associated diarrhea, irritable bowel syndrome and food allergies. Further, recent clinical and nutritional studies have encountered the function of specific strains in energy metabolism and thereby have opened up new angles on their exploitation.

Keywords: probiotics, gut micobiota, health, diseases.

Presented in : Society for Anti Aging Aesthetic Regenerative Malaysia 2015

INTRODUCTION

The dominant microbial compositions of the intestine have been shown to be stable over time during adulthood, and the microbial patterns are unique for each individual. However, there are numerous external factors that have potential to influence the microbial composition in the gut as host genetics, birth delivery mode, diet, age, antibiotic treatments and also, other microorganisms as probiotics.

During birth and rapidly thereafter, microbes from mother and also, from the environment colonize the gastrointestinal tract of the infant. The microbiota of a newborn develops rapidly and is initially strongly dependent on the mother's microbiota, the mode of birth, environment at birth and hygiene, subsequently, it is influenced by feeding practices and the environment of the infant. The bacteria identified include members of the genera *Bifidobacterium*, *Enterococcus*, *Clostridium*, and *Enterobacter* among others.

The intestine is one of the main surfaces of contact with exogenous agents (viruses, bacteria, allergens) in the human body. It has a primary role in the host defense against external aggressions by means of the intestinal mucosa, the local immune system, and the interactions with the intestinal microbiota (resident and in transit bacteria). Gut microbiota influences human health through an impact on the gut defense barrier, immune function, nutrient utilization and potentially by direct signaling with the gastrointestinal epithelium.

A balance among microbial groups present in human gut is crucial for maintaining health. When this balance is disturbed, the host–microbe relationship can progress towards a disease state. In addition, it has been described that microbiota deviations are associated with enhanced risk of specific diseases including inflammatory bowel diseases, irritable bowel disease and antibiotic associated diarrhea and it has been associated with allergy, obesity and diabetes. Thus, the maintenance of microbiota equilibrium is important to preserve and to promote health.

The intestinal microbiota can be identified as an active "organ", which is involved in different processes such (i) the improvement of nutrient bioavailability and degradation of non-digestible dietary compounds, (ii) the supply of new nutrients, and (iii) the removal of harmful, toxic and non-nutritional compounds. These metabolic functions have important implications in human health and nutrition, although they depend on the composition of the microbiota and its complex interactions with the diet and the host.[1-3]

Metabolic and Nutritional Activity of the Microbiota

Gut microbiota is essential for processing dietary polysaccharides affecting energy harvest from the diet. It provides additional energy in the form of short-chain fatty acids including acetate, propionate and butyrate.

Several members of the intestinal microbiota can produce vitamins and provide them to the host, mainly vitamin K and also, some vitamins B. The significance of the microbiota in salvaging energy and producing vitamins is most clearly seen in germfree animals. Compared to conventional animals, germ-free animals require 30% more energy in their diet and supplementation of which with vitamins K and B is mandatory to maintain their body weight. The absorption of calcium, magnesium and phosphorus is also improved by carbohydrate fermentation and production of short chain fatty acids (SCFA), and pH reduction. In addition, the acidification of intestinal environmental also inhibits the development and colonization of pathogens or undesirable bacteria, as well as the production toxic elements derived from their metabolism (ammonia, phenol compounds, amines, etc.). The microbiota is reported to contribute to human protein homeostasis. At least some requirements for amino acids are met by microbial synthesis. In contrast, fermentation of amino acids may lead to the production of a variety of toxic substances such as tumor inducers and promoters.[1-3]

Immune System

The intestinal immune system constitutes the primary immune organ of the human body and an essential element of the host defense against pathogenic microorganisms.

The intestinal microbiota provides an important stimulus for the immunity development of the host immune system and regulates innate and adaptative immunity. At birth, the immune system is immature and develops upon exposure to the intestinal microbiota; increasing the number of Peyer's patches and immunoglobulin producing cells. The innate immune system allows the host to sense a concrete microbial environment in order to promote the release of signaling molecules (cytokines and chemokines) and initiate the immune response. In general, it is considered that epithelial and monocytic cells recognize the signature molecules called pathogen-associated molecular patterns (PAMPs), which activate the host defense mechanisms. In contrast, the commensal bacteria share signature molecules called microbe-associated molecular patterns (MAMPs), which do not tiger pro-inflammatory responses.

Role in Energy Harvest

Gut microbiota is essential for processing dietary polysaccharides affecting energy harvest from the diet. It provides additional energy in the form of short-chain fatty acids including acetate, propionate and butyrate. Acetate is taken up primarily by peripheral tissues and can also be used by adipocytes for lipogenesis. Propionate is an important precursor for gluconeogenesis in theliver and the butyric acid is mostly metabolized by the intestinal epithelium, as a main energy source for the intestinal epithelium providing between 60 and 70% of all the energy, is important in maintaining mucosal health in the colon. Moreover, microbiota has been reported to be involved in fat storage in the host. It has been reported a 60% of increase in body fat content in germ-free rats upon colonization with conventional microbiota and also, cholesterol-lowering microbes from intestinal microbiota.

Probiotics were originally used to influence both animal and human health through modulation of the intestinal microbiota. At present, the specific live microbial food ingredients and their effects on human health are studied both within food matrices and as single or mixed culture preparations. In general, for microorganisms to be considered as probiotics, the following criteria need to be fulfilled: i) It should be isolated from the same species as its intended host; ii) It should have a demonstrable beneficial effect on the host, iii) It should be non-pathogenic nontoxic, and free of significant adverse side effects; iv) It should be able to survive through the gastrointestinal tract, v) It should be stable during the intended product shelf life and contain an adequate number of viable cells to confer the health benefit and vi) It should be compatible with product format to maintain desired sensory properties; and labeled accurately.

PROBIOTIC MECHANISMS OF ACTION

Some of the beneficial effect of probiotic consumption include: the improvement of intestinal tract health by means of regulation of microbiota and stimulation and development of the immune system, synthesizing and enhancing the bioavailability of nutrients; reducing symptoms of lactose intolerance and reducing risk of certain diseases. Several mechanisms of action have been proposed to explain the beneficial effects of probiotics. The mechanisms by which probiotics exert their effects are largely unknown, but may involve modifying gut pH, antagonizing pathogens through production of antimicrobial compounds, competing for

pathogen binding, receptor sites, nutrients and growth factors, stimulating immunomodulatory cells, and producing lactase.

Probiotics have specific targets of action and they are probiotic dependent and also, different bacterial strains may differ with respect to their effects on health. In addition, it must be taken into consideration that these mechanisms may be multi-factorial processes and also, dependent on each probiotic strain that may have specific functions affecting to the health host. Considering their possible targets, probiotics would be designed and applied in specific diseases based on their characteristics and health-promoting effects. Different levels of host-microbe interaction can be distinguished:

i) **microbe-gut epithelium interaction:** adhesion to mucosal and epithelial cells, stimulation of mucus secretion and enhancing the production of defensive molecules such as mucins, increasing and reinforcing barrier function, innate immune function, reducing the secretory and inflammatory consequences of bacterial infection and improving gut motility;

ii) **microbe-immune system interaction:** Immune-modulation and regulation of immune responses beyond the gut and finally,

iii) **microbe-microbe interaction:** Modify microbiota to suppress and inhibit pathogens; prevention of adhesion, establishment and/or replication of pathogens in the gastrointestinal tract; Secretion of antimicrobial substances and compounds, compete for nutrients necessary for pathogen survival and anti-toxin effects.

The general mechanisms by which probiotics may have an effect can be divided into different categories: normalization of microbiota, modulation of immune response, and metabolic functions.

Adhesion

Adhesion to the intestinal mucosa is regarded as a prerequisite for colonization and is an important characteristic related to the ability of strains to modulate the immune system. Thus, adhesion has been one of the main selection criteria for new probiotic strains. Many different intestinal mucosa models have been used to assess the adhesive ability of probiotics; among them the adhesion to human intestinal mucus has been widely used and good correlations have been reported with other models.

The adhesion levels of the probiotic and pathogens strains showed a great variability and are dependent on each strain, species and genus. The adhesion properties of probiotics widely

vary depending on the strain and high adherence ability in one strain not always guarantee an *in vivo* persistence and protective effect and this should always be corroborated by studies in animal models and humans. In *in vitro* trials, the probiotic properties have mainly been tested alone or in combination with yoghurt bacteria such as *L. delbrueckii* and *L. acidophilus* but rarely combined with other probiotics. However, some studies are available on the interactions of probiotics regarding adhesion properties in the intestinal mucus system.

Antimicrobial Substances

In general, the antimicrobial metabolites produced by lactic acid bacteria can be divided into two groups: (i) low molecular mass compounds (bellow 1,000 Da) such as organic acids, which have a broad spectrum of action, and (ii) antimicrobial proteins, termed bacteriocins (>1.000 Da), which have a relatively narrow specificity of action against closely related organisms and other Gram-positive bacteria.

The acids secreted in the fermentative metabolism of carbohydrates by probiotics have been considered to be the main antimicrobial compounds responsible for their inhibitory activity against pathogens. Bacteriocins are proteins or protein complexes that show bactericidal activity against bacterial species that are closely related to the producer species. Most of the studies related to the characterization of bacteriocins or bacteriocin-like compounds from lactic acid bacteria have been focused on species of the genera *Lactobacillus, Pediococcus* and *Enterococcus*, because of the diversity of their species and their potential applications as natural preservatives in foods.

Immunomodulation

Recent studies have shown that intake of specific probiotics can be effective in preventing and repairing damage of the intestine. Probiotics are able to stimulate, as well as regulate natural and acquired immune responses by interacting with the mucosa-associated lymphoid tissue. This interaction has broad-reaching significance to human health and could impact infectious diseases, some forms of cancer, allergic disease, autoimmune disorders, and also, a range of intestinal inflammatory diseases. Probiotics can bind to receptors on the surface of epithelial cells, inducing humoral and cellular immune response that could be directed both to anti-inflammatory and pro-inflammatory directions. There is a large variation in the induced response among strains and species but there are increasing evidences that some probiotics can stimulate a protective immune response sufficiently to enhance resistance to microbial pathogens. Animal and human studies show that different strains of probiotics can have

different effects on the immune system. Effects also are dependent on dose and on immune status of the host. Some animal studies have demonstrated reduced gut permeability associated with cow's milk feeding and inflammatory bowel disease.

Probiotics may mediate these effects in several ways: by inhibiting damage to intestinal cell junctions ; improving cell growth and survival ; inducing mucin secretion ; decreasing bacterial adhesion ; and secreting repair factors and nutrients (e.g., short-chain fatty acids, polyamines, nitric acid, and stimulating production of secretory immunoglobulin A [IgA]).[1,2,4,5]

PROBIOTIC IMPACT ON GUT HEALTH AND DISEASE:

The primary clinical interest in the application of probiotics has been in the prevention and treatment of gastrointestinal infections and also, related to the management of gastrointestinal infections caused by pathogenic microorganisms as *H. pylori*, *E. coli*, *Salmonella*, *Clostridium*. Microbiota deviations have been associated with enhanced risk of specific diseases such as acute gastroenteritis, atopic diseases, obesity and even autism. Therefore, modulation of an unbalanced indigenous microbiota forms the rationale of probiotic therapy. During the last few decades, a large number of studies have been conducted to assess probiotic microorganisms, using different formula and with specific purposes of preventing or treating diseases. Pathogens may alter the intestinal permeability while probiotic strains could prevent damage and repair and restore the mucosal integrity, increase the epithelial resistance against pathogens and induce cell proliferation. In addition, specific probiotic strains have been shown to exert a protective effect against acute diarrhea, rotavirus diarrhea, and antibiotic-associated diarrhea, as well as *Helicobacter pylori* infection, and they alleviate symptoms of gastrointestinal diseases such as irritable bowel syndrome. The use of probiotics should be further investigated for their possible benefits but also side-effects if any.

***Helicobacter pylori* Infection**

Urease, an important product produced by *H. pylori*, hydrolyses urea to ammonium, leading to increased pH in the stomach which promotes colonization of the microorganism. It has been demonstrated that the eradication of *H. pylori* from the stomach requires a combination of therapies. Antibiotics (e. g. amoxicillin, clarithromycin, or nitroimidazoles) are used together with acid suppression drugs (proton pump inhibitors or H2-receptor antagonists),

in triple or quadruple combinations. Treatment aimed at *H. pylori* eradication has been reported to give rise to ecological disturbances with suppression of normal microbiota and emergence of antibiotic-resistant microbes. Several *in vitro* and *in vivo* studieson the role of probiotics in the treatment of *H. pylori* infections have been performed during recent years and they have demonstrated that the probiotic strains inhibit the growth or the attachment of *H. pylori* by means of organic acid production, antimicrobial substances of proteinaceus-origin, competitive inhibition for the binding sites to mucus-producing cells, and immunomodulation.

It has been observed that *Lactobacillus salivarius* inhibits the colonization of *H. pylori* and *L. salivarius* given after *H. pylori* implantation also eliminates the colonization by *H. pylori* in mice. Clinical studies have tested and reported the potential use the use of probiotics as a supplement to antibiotic therapy for *H. pylori* eradication. In these studies, the use of probiotics decreased the side effects of antibiotics, improved patient compliance with taking the prescribed therapy, and increased the rate at which *H. pylori* was eradicated. No study could demonstrate the eradication of *H. pylori* infection by probiotic treatment but long-term consumption of probiotics may have a favorable effect on *H. pylori* infection in humans, particularly by reducing the risk of developing disorders associated with high degrees of gastric inflammation.[4-7]

Diarrhea

The ability of probiotics to decrease the incidence or duration of certain diarrheas is the most substantiated health effects of probiotics. The majority of studies on effect of probiotics in diarrhea have been related to the treatment of acute infectious diarrhea in children. A number of specific strains, including *Lactobacillus* GG, *Lactobacillus reuteri*, *Lactobacillus casei*, *Saccharomyces boulardii*, *Bifidobacterium* strains and others, have been demonstrated by controlled clinical trials to decrease the severity and duration of acute diarrhea. In addition, these probiotics have been also effective in the treatment of other diarrheas including travellers' diarrhea and diarrhea disease in young children caused by rotaviruses. Administration of probiotics may reduce the duration of acute diarrhea in children by approximately 1 day. Meta-analysis concludes that these probiotics are safe and effective.

Antibiotic- and *Clostridium difficile*-Associated Diarrhea

Some probiotic strains have shown to exert a protective effect specifically against *Clostridium difficile* diarrhea and antibiotic associated diarrhea. A common complication of treatments with antimicrobial agents is the development of antibiotic associated diarrhea (AAD) in 5–25% of patients. Antimicrobial treatment alters the ecological balance of the healthy microbiota, which can result in diarrhea and emergence of some pathogens like *C. difficile.* The immune response of the host to *C. difficile* toxins has further been shown to be a determinant of susceptibility.[5,6]

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a chronic and recurrent inflammation generally affecting the colon or the small intestine and includes ulcerative colitis, pouchitis, and Crohn's disease. The etiology of IBD remains unclear, but genetic predisposition and alterations in microbiota are involved. There is evidence that the immune system reacts abnormally towards the endogenous microbiota. Patients with inflammatory bowel diseases have higher levels of specific bacteria as *Bacteroides* attached to epithelial cells than healthy people. Therapeutic manipulation of the normal gastrointestinal microbiota using probiotics, alone or indifferent combinations has been regarded as a treatment option. Researchers are assessing the use of probiotic strains and combination in the treatment of inflammatory bowel diseases although few studies are available in IBD in general andalso, in ulcerative colitis, chronic pouchitis and Crohn's disease. The best results have been obtained so far on ulcerative colitis and chronic pouchitis. A pilot study suggested that a fermented milk containing *B. breve*, *B. bifidum* and *L.acidophilus* can be useful to induce remission in ulcerative colitis patients with mild disease. The VSL#3 probiotic mixture also has been proved highly effective for maintaining remission of chronic pouchitis.[6-8]

Allergic Disease

Microbiota aberrancies have been related to the development of allergic diseases. A reduced ratio of bifidobacteria to clostridia has been detected in infants developing atopy, and allergic patients have been shown to be more often colonized with *Clostridium* and *Staphylococcus*, and have fewer *Enterococcus* and *Bifidobacterium* than do non-allergic individuals. Alterations in gut microbiota composition may precede the development and manifestation of atopic diseases. Early colonization of *Escherichia coli* has been associated with higher risk for developing eczema, and that of *Clostridium difficile* with eczema, recurrent wheeze, and

allergic sensitization in infancy. The hygiene hypothesis supports the rapid increase of atopy related to lower microbe exposure at early life and subsequent lower number of infections during early life. Recent studies reported microbiota differences between allergic and healthy infants in countries with high and low allergy prevalence. These changes on microbiota may be counter-balanced by probiotic bacteria. Positive clinical effects of probiotics on the prevention and treatment of atopic diseases have been reported. The effectiveness of *L. rhamnosus* GG in the prevention of atopic dermatitis has been reported in randomized, controlled trials. *L. rhamnosus* GG was given to pregnant women for four weeks prior to delivery, then to newborns at high risk of allergy for six months resulting in a significant reduction in early atopic disease. This study illustrates the potential for probiotic microorganisms to modulate the immune response and prevent allergic diseases. In other clinical studies with infants allergic to cow's milk and also, atopic dermatitis was alleviated by ingestion of probiotic strains *L. rhamnosus* GG and *B. lactis* Bb12. [6-8]

Cancer

Evidence from a wide range of sources supports the view that the colonic microbiota may be involved in the etiology of colorectal cancer (CRC). This hassled to an intense interest in factors that can modulate the gut microbiota and their metabolism. The ingestion of probiotics, prebiotics or combinations of both (synbiotics) represents a novel new therapeutic option because they act to alter the intestinal microbiota by increasing concentrations of beneficial bacteria and reducing the levels of pathogenic micro-organisms. This strategy inhibits the development and progression of neoplasia via mechanisms including; decreased intestinal inflammation, enhanced immune functionand anti-tumorigenic activity, binding to potential food carcinogens including toxins found in meat products, and a reduction in bacterial enzymes such as beta-glucuronidase which hydrolyse precarcinogenic compounds.

Several experimental animal studies clearly demonstrated a protective effect of probiotics such as some *Lactobacillus* and *Bifidobacterium* strains, or the combination of prebiotics and probiotics on the establishment, growth, and metastasis of transplantable and chemically induced tumors. Human intervention trials to confirm these animal studies are intrinsically difficult because of the natural history of the disease (difficulty in selecting subjects at high risk and requirement of long-term follow-up). A 4-year study found that *L. casei* Shirota decreased the recurrence of atypical colonic polyps [171]. Further studies are needed to clarify the potential role of probiotic in the prevention of CRC.[7,8]

Obesity

Obesity is viewed as one of the major current public health problems and its impact is the highest in children, contributing to significant morbidity in adulthood. The developments of metabolic complications associated with obesity during childhood track into adulthood and increase the risk for type 2 diabetes and early cardiovascular diseases. Risk factors for infant obesity include diet, low socioeconomic status, maternal obesity, rapid
infancy weight gain, and decreased physical activity. In addition to all of these risks, some reports have suggested that the gut microbiota is an important factor affecting energy disposal and storage in adipocytes. In addition, a recent report showed that infants with high numbers of the *Bifidobacterium* and low numbers of *Staphylococcus* may be protected from excess weight gain during later life. Since *Bifidobacterium* genus and species are prevalent in breast-fed infants, it has been suggested why breastfed babies have been found to be at lower risk for later obesity. Thus, these findings open new gates to prevent obesity due obese children often become obese adults and maternal obesity also over-nourishes the infants, thereby programming adult size and health with a heightened risk of obesity later in life. In addition, it has been reported how diets based on a high intake of protein and/or low intake of carbohydrate or low fat consumption may alter microbial composition and activity in the large intestine and thus impact on gut health. Microbiota modification by use of probiotics may offer new directions for preventive and therapeutic applications in reducing the risk of overweight and obesity.[7,8]

CONCLUSIONS

The understanding on intestinal microbiota, nutrition, immunity and genetics in health and disease has increased in last year's. This information will help develop new probiotic strains with disease-specific functions. Thus, an appropriate selection of probiotic strains is the basic for the further development of new probiotic products as well as for planning human clinical trials. *In vitro* studies are important to assess the safety and efficiency of probiotics and also, they are useful to expand the knowledge of specific properties of tested strains. Rational selection and validation of probiotic strains should be based on evidence obtained in *in vitro* and *in vivo* models with a reliable predictive value or function, and followed by studies in humans.

Recent advances have been made in the understanding of when to use probiotics and how they impact specific pathological states. The concept that probiotic may have benefits in a multitude of disorders and they exert health promoting effects has been reported. In addition, recent reports support the use of probiotics in the prevention and treatment of a number of dysfunctions including atopic diseases, immune disorders, obesity, and diabetes.

Refference

1. Collado MC, Isolauri E, Salminen S, Sanz Y. The Impact of Probiotic on Gut Health. Current Drug Metabolism.2009.10:68-78.

2. Zucotti GV, Menegghin F, Raimondi C, Dillilo D, Agostoni C, Giovannini M. Probiotics in clinical practice: an overview. J Int Med Res.2008;36 Syppl 1:1A-53A.

3. Thoreux K, Owen R, Schumacker DL. Functional foods, mucosal immunity and aging: effects of probiotics on intestinal immunity in young and old rats. Communicating Curret Research and Educational Topics and Trends in Applied Microbiology.

4. Snelling AM. Effects of Probiotics on the gastrointestinal tract. Curr Opin Infect Dis. 2005 Oct;18(5):420-6.

5. Bodera P, Chcialowski A. Immunomodulatory Effect of Probiotic Bacteria. Recent Patents on Infflammation & Allergy Drug Discovery.2009.3:58-64.

6. Delcenseria V, Martel D, Lamorouex M, Amiot J, Boutin Y, and Roy D. Immunomodulatory effects of probiotics in the intestinal tract. Curr Issues Mol Biol.2010.10:37-54.

7. Snaz Y, Nadal I, Sanchez E. Priotics as driugs againtz human gastrointestinal infections. Recent Pat Antiinfect Drug discov. 2007 Jun;2(2):148-56.

8. Magrone T, Jirillo E. The interaction between gut microbiota and age-related changes in immune function and iflammation. Immunity and Ageing.2013:10:31.