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Review Article

PROBIOTICS: THE UNDERVALUED CONQUERORS

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ABSTRACT

Probiotics or "beneficial microorganisms" have recently acquired the status of blockbusters in the pharmaceutical industry. Despite being commercially available, little is known about the magnitude to which they can contribute to various disease conditions such as inflammatory bowel disease, allergy, immunity, etc. this review dwells into the various mechanisms of actions of probiotics and summarizes the numerous benefits of these daily used products, including the recent advances. It also emphasizes on the possible unfavorable consequences, which can result due to their extensive use.

Keywords: Probiotics, Allergy, Immunity, Inflammatory bowel disease.

INTRODUCTION

Humans stay in association with innumerable microorganisms located in the gut, mouth and on the skin as commensals. A symbiotic relationship exists between the two wherein the microorganisms play a crucial role in modulation of the host immunity in return of obtaining shelter and nutrition from the host. This is in accordance with the hologenome theory of evolution [1].

An ornate assortment of flora consisting of almost 400 species resides in the gastrointestinal tract, which regulates the host immunity, nutrition and intestinal homeostasis. They play an eminent role in maintaining the enterohepatic circulation of the exogenous compounds via the release of certain enzymes such as glucuronidase and sulfatase and also in the fermentation of dietary fibers and dietary carcinogens [2]. It is also involved in the synthesis of vitamins such as vitamin K and folate. The role of this flora in regulating our immunity by modulation of nucleotide-binding oligomerization domain 2 and factors such as transcription factor nuclear factor (NF)-Kb [3]. Alteration of this flora by factors such as antibiotics can present in a diverse range of symptoms resulting in deleterious consequences.

Probiotics or "beneficial microorganisms" have become a new trend in this era of modern pharmacology. They have acquired one of the largest markets of commercial interest. They have so far been considered to be beneficial for the consumers, despite the lack of elaborate clinical endpoints during research practices [4]. In the last three decades, the US market of functional food including the prebiotics and probiotics has grown to the wavering number of 33 billion US $[5].

HISTORY

The origin of the word probiotic originates from Greek etymology where "*βιωτικός*" means "life." The "pro" has been suggested to be derived from Latin word which means "for" the human gut is inhabited with a vast variety of microbes, which vary at the extremes of age. The assembly of genomes from these microorganisms constitutes the intestinal microbiome [6]. The most commonly derived probiotics come from two groups, *Lactobacillus* or *Bifidobacterium*.

The evolution of probiotics can be traced back to prehistoric times when the food bacterial content was much more as compared to now. This was probably responsible for the discovery of fermentation of beer and wine [7]. Lilly and Stillwell used the term "probiotic" for the first time for the chemicals that were produced by organisms and further enhanced the growth of others [8]. In 1997, Eli Metchnikoff, Father of Natural Immunity, brought forth the concept of ingestion of bacteria in order to sustain the normal flora of the human gut [9]. Nurturing the idea that lactic acid bacillus can be capable of prolonging life he formulated a diet of milk fermented with a bacterium called "burgarium bacillus."

However, with the identification of microbes, and the establishment of their association with diseases, the use of these probiotics became limited. In addition, the industry's production of further refined products and the need to increase the shelf life of the products resulted in the replacement of these bacteria with enzymes.

PROBIOTICS AND PREBIOTICS: DIFFERENCE IN THE TERMINOLOGIES

Probiotics are primarily live beneficial organisms administered in order to restore the normal gut flora or to provide additional health benefits. Contrarily, the presence of an appropriate flora is a prerequisite for the prebiotics to act. Synbiotics applies to a combination of a pro and prebiotics Table 1.

WHEN TO CALL IT A PROBIOTIC

Following points innumerate the requirements to evaluate probiotics in food as per the 2002 WHO/FAO guidelines for Evaluation of Probiotics in food

1. With the help of the genotype and phenotype testing, the genus, species and strain need to be established.
2. *In-vitro* tests are to be done to evaluate the beneficial effect and gain knowledge regarding the particular strain.
3. It should be ensured that the particular strain is safe and henceforth can be delivered to human without any contamination.
4. Adequate *in-vivo* studies in humans and animals to substantiate the *in-vivo* effects.
5. General health claims should be permitted on food containing probiotics, provided they contain adequate information regarding their effect, and the label contains data regarding the dose, shelf life and storage instructions [10].

Even among the bacteria that come under the category of "generally regarded as safe," the Working Committee recommended that probiotics can be recognized if the following criteria are fulfilled at the minimum:

- The antibiotic resistant pattern is ascertained
- The metabolic activities and side-effects have been assessed
- Epidemiological surveillance of the adverse events in humans
- If the known species, is known to be responsible for production of a toxin, it has been tested for the same
- If the known species has hemolytic potential, it has been determined.

Table 1: Important terminologies

S. no	Term	Definition	Examples
1.	Probiotic	Live viable microorganisms which when administered in adequate amounts, confer health benefits to the host	*L. acidophilus*
2	Prebiotic	Non digestible chemical substances that induce the growth or activity of commensal microorganisms	FOS, XOS
3	Synbiotic	Mixture of probiotic and prebiotic that has synergistic action and confers physiological health benefits to the host	Bifidobacteria and FOS
4	Antibiotic	Agent that kills microorganisms	Penicillin

FOS: Fructooligosaccharides, XOS: Xylooligosaccharides, *L. acidophilus: Lactobacillus acidophilus*

FUNCTIONS OF PROBIOTICS

Though not yet fully understood, several mechanisms have been proposed to contribute to the function of probiotics [11]. Although the overall picture may be similar, each strain exerts its effects via an individual profile of cytokines, which is yet to be elucidated [12]. The job of microbiota can be roughly classified into 4 aspects:

1. Nutritional
2. Microbiological
3. Physiological
4. Immunological.

USES

Probiotics have the potential of being utilized in abundant medical condition. Their use in some conditions has been definitely proven to be useful, there are still conditions where their use is still debated due to a lack of a plausible mechanism of action. However, following are some of the conditions where the use of probiotics has been emphasized Table 2.

In inflammatory bowel disease

Probiotics have been recently found to be of utmost importance in the treatment of inflammatory bowel disease (includes crohn's and ulcerative colitis). Apart from their known positive effect on the epithelial dysfunction and mucosal immune system [16], they play an inevitable role in interrupting the activity of the effector T cells, which justifies their predominant role in allaying the inflammatory changes [17].

However, a meta-analysis of eight randomized placebo-controlled trials failed to validate the usefulness of probiotics for maintenance of remission and preventing a further relapse of Crohns disease [18].

Various mechanisms proposed: [19,20]

- Production of bactericidal substances
- Competing with the bacteria for adhering to the intestinal epithelium
- Enhance innate immunity
- Regulation of intestinal homeostasis by enhancing defensive mechanisms.

Irritable bowel disease

Irritable bowel disease has a wide plethora of manifestations such as bloating, constipation, diarrhea, etc. However, what bothers most is the abdominal pain due to the visceral hyperalgesia following colonic distension. This has been explained by different postulates such as alteration in neurotransmitters such as serotonin, hormone such as corticotropin-releasing hormone and even at the level of anterior cingulate gyrus [21]. One of the animal studies demonstrated that administration of *Lactobacillus* paracasei had a significant influence on the visceral hypersensitivity in mice [22]. Through their multimodal action on cytokines such as interleukin (IL-10). IL-12, tumor necrosis factor-α (TNF-α) and their effect on colonic motility also they have suggested to be highly beneficial in these patients [23].

Probiotics in necrotizing enterocolitis

NEC is a serious condition seen in premature infants whereby a part of the bowel undergoes necrosis resulting in the death of the infant. Though the exact pathogenesis remains to be elucidated, a number of theories have been put forth. One of the plausible theories includes an alteration of the gut flora, which may result in NEC [24]. The surplus growth of Gram-negative bacteria with the dearth of Gram-positive bacteria has been contemplated to be responsible for the insult to the bowel [25]. According to an analysis of about twenty-four trials, probiotic supplementation with *Lactobacillus* and *Bifidobacterium,* prevented severe NEC in premature infants [26]. However, another study including 271 infants found that Saccharomyces boulardii did not have an overall effect on the mortality, but, however, it resulted in a reduction of the incidence of sepsis [27]. The mechanism of their beneficial effect, as hypothesized in a review, is the restoration of the normal flora which will further enhance their anti-inflammatory effect and also competing with the pathogenic bacteria for the same environment [28].

Probiotics for infectious diarrhea

Diarrhea comes from a Greek word διαρροια which means flowing through. Diarrhea accounts for one of the foremost causes of morbidity and mortality in children. Treatment profile of various types of diarrheas involves treating the cause along with adequate rehydration therapy. However, the role of probiotics as a line of treatment still remains to be determined. Numerous animal studies have validated the role of interactions of commensals in the gut with the host in upholding a normal environment and combating various pathogenic microbes [29]. *Lactobacillus rhamnosus* strain HN001 when fed to mice, enhanced immunity against *Salmonella typhimurium* [30]. *L. rhamnosus* strain can bind to aflatoxin B, and can help in the removal of toxins [31]. Another perspective provided by Coakley *et al.* Advocates the production of conjugated linoleic acid by a specific strain of *Bifidobacterium* to be an additional mechanism contributing to its role on the road to recovery [32]. In conditions such as chemotherapy or radiotherapy induced enteritis, administration of probiotics can mitigate the degree of occurrence of symptoms [33]. When compared to placebo, administration of *Lactobacillus* in children can result in significant reduction of stools on day 2 [34]. Further, a meta-analysis of 63 studies with approximately 8000 participants concluded the overall role of probiotics in kerbing the duration as well as the frequency of infectious diarrhea [35]. Following are some mechanisms involved in their role in infectious diarrhea [36] (Fig.1).

Effect of probiotics on lipid profile

Though the effect of probiotics on overall lipid profile still remains controversial, their predominant effect is suggested to be on cholesterol levels. By acting on the various steps of cholesterol metabolism, they result in an inhibition in absorption and synthesis of cholesterol and further causing its movement from the liver to plasma. Through fermentation of the carbohydrates, they lead to the production of short chain fatty acids which possess cholesterol lowering potential [37]. The bacterial activity can also inhibit the conjugation of bile acids thus promoting their excretion. A study was done on diabetic Type 2 patients suggested a non-significant effect on the triglycerides [38]. However, the magnitude to which this effect can be seen is subjected to strain specificity. In the animal studies, *Lactobacillus acidophilus* strain - *L. acidophilus* RP32 exhibited a significant role in constraining the rise in cholesterol on pigs [39].

Table 2: Functions of probiotics

Nutritional	Microbiological [13]	Physiological	Immunological
Increase level of Vitamin B1 and B2[14] Antioxidant[15] Production of conjugated linoleic acid Improvement in lipid profile	Competing for the same niche Competition for the nutrients Production of toxins Production of reactive superoxide radicals Alteration of pH	Enhance GI Transit	Maintain the integrity if the gut barrier Decrease the exposure of antigens to the immune cells

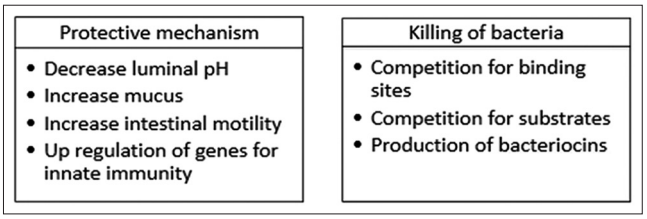

Fig. 1: Mechanism of action in infectious diarrhoea

Role in allergy

Allergy is an immune reaction which may be characterized by hyper-responsiveness to stimuli resulting in a magnanimous release of cytokines in the body. Type 1 hyper sensitivity may manifest in multitude type of reactions such as eczema, asthma, rhinitis, etc. The interaction between the intestinal microbiota and microorganisms forms the basis of modulating the immune response via T cells [40]. With respect to the asthmatic child having concomitant allergic rhinitis, a down-regulation of certain cytokines such as TNF-a, interferon-γ by probiotics plays a predominant role in kerbing the immune response [41]. It has also been implicated in causing a reduction of episodes of allergic rhinitis [42].

Scope of probiotics in psychiatry

The gut microbiota exits in harmony with the host in the gut. A discord in various scenarios can result in the onset of psychiatric disorders [43]. Interestingly, if one looks at it from a different perspective, the gut microflora can have substantial effects on the brain. The bacterial toxins may mimic the endogenous transmitters or may cross the blood-brain barrier and result in inflammatory changes [44]. The alteration in the stress response by microflora can be an essential contributor to the onset of psychiatric ailments [45]. Their antioxidant effects is being tried to be used in mood disorders [46].

SAFETY OF PROBIOTICS: AN UNANSWERED QUERY

A long-standing doubt in the minds of many is whether ingestion of probiotics can prove to be harmful to the host or not. The fact that they are live microorganisms which are taken in has resulted in a conflict of interest among many. In immunocompromised patients, it becomes even more important to weigh the benefits against the risks. The main cause of alarms is 3 theories which have been associated with probiotics [47].

1. They can contribute in any way to occurrence of disease
2. The effect of the toxins on the gut
3. Role in transfer of antibiotic resistance [48].

In one of the studies, *Lactobacillus* GG administration in pregnant females resulted in an augmentation in the number of episodes of wheezing bronchitis [49].

ROLE OF PROBIOTICS IN TRANSFER OF ANTIBIOTIC RESISTANCE

Horizontal gene transfer is the process whereby there is swapping of the genetic material between neighboring bacteria where the plasmids, transposons act as vectors. With the increasing evidence of antibiotic resistance for several strains of *Lactobacilli*, the potential role of probiotics in the spread of horizontal antibiotic resistance has been questioned. A recent review by Sharma *et al*. highlights the possibility of transference of resistance of genes such as tetracycline resistance gene from probiotics to the pre-existing gut flora [50].

RECENT ADVANCES

Off lately the molecular mechanisms of action of probiotics have been under research. The phosphatidylinositol3 kinase/AKT pathway is involved in allaying apoptosis of cardiac cells in obese rats, which can be extended in clinical practice [51]. Because of its known effect on lipid profile, the idea has been further extended to its potential use in metabolic syndrome [52]. VSL#3 is the most extensively studied mixture of eight strains of bacteria which has been tested on various animal models and is now being extensively used in human trials [53]. The use of Ma-Pi 2 macrobiotic diet has recently ignited a worldwide interest for its application in diabetic patients [54]. Consisting of 12% protein, 18% fat and 70% carbohydrate and being rich in probiotics and prebiotics it has become one of the valuable interventions to generate a positive effect on the conglomerate of lab parameters such as lipid profile and glucose [55]. Another promising approach is modulation of the enteric microflora in non-alcoholic fatty liver disease [56].

REFERENCES

1. Zilber-Rosenberg I, Rosenberg E. Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. FEMS Microbiol Rev 2008;32(5):723-35.
2. Gorbach SL. Microbiology of the Gastrointestinal Tract. Galveston, TX: University of Texas Medical Branch at Galveston; 1996.
3. O'Hara AM, Shanahan F. The gut flora as a forgotten organ. EMBO Rep 2006;7(7):688-93.
4. Rijkers GT, de Vos WM, Brummer RJ, Morelli L, Corthier G, Marteau P. Health benefits and health claims of probiotics: Bridging science and marketing. Br J Nutr 2011;106(9):1291-6.
5. Figueroa-González I, Quijano G, Ramírez G, Cruz-Guerrero A. Probiotics and prebiotics – Perspectives and challenges. J Sci Food Agric 2011;91(8):1341-8.
6. Salazar N, Arboleya S, Valdés L, Stanton C, Ross P, Ruiz L, *et al*. The human intestinal microbiome at extreme ages of life. Dietary intervention as a way to counteract alterations. Front Genet 2014;5:406.
7. Bengmark S. Ecological control of the gastrointestinal tract. The role of probiotic flora. Gut 1998;42(1):2-7.
8. Gupta V, Garg R. Probiotics. Indian J Med Microbiol 2009;27(3):202-9.
9. Gordon S. Elie Metchnikoff: Father of natural immunity. Eur J Immunol 2008;38(12):3257-64.
10. FAO/WHO. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food; 2002.
11. Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A. Probiotic mechanisms of action. Ann Nutr Metab 2012;61(12):160-74.
12. Delcenserie V, Martel D, Lamoureux M, Amiot J, Boutin Y, Roy D. Immunomodulatory effects of probiotics in the intestinal tract. Curr Issues Mol Biol 2008;10(1-2):37-54.
13. Vandenplas Y, Huys G, Daube G. Probiotics: An update. J Pediatr (Rio J) 2015;91(1):6-21.
14. Fabian E, Majchrzak D, Dieminger B, Meyer E, Elmadfa I. Influence of probiotic and conventional yoghurt on the status of vitamins B1, B2 and B6 in young healthy women. Ann Nutr Metab 2008;52(1):29-36.
15. Fabian E, Elmadfa I. The effect of daily consumption of probiotic and conventional yoghurt on oxidant and anti-oxidant parameters in plasma of young healthy women. Int J Vitam Nutr Res 2007;77(2):79-88.
16. Fedorak RN. Understanding why probiotic therapies can be effective in treating IBD. J Clin Gastroenterol 2008;42 Suppl 3:S111-5.
17. Boirivant M, Strober W. The mechanism of action of probiotics. Curr Opin Gastroenterol 2007;23(6):679-92.

18. Rahimi R, Nikfar S, Rahimi F, Elahi B, Derakhshani S, Vafaie M, *et al.* A meta-analysis on the efficacy of probiotics for maintenance of remission and prevention of clinical and endoscopic relapse in Crohn's disease. Dig Dis Sci 2008;53(9):2524-31.
19. Vanderpool C, Yan F, Polk DB. Mechanisms of probiotic action: Implications for therapeutic applications in inflammatory bowel diseases. Inflamm Bowel Dis 2008;14(11):1585-96.
20. Yan F, Polk DB. Probiotics: Progress toward novel therapies for intestinal diseases. Curr Opin Gastroenterol 2010;26(2):95-101.
21. Kanazawa M, Hongo M, Fukudo S. Visceral hypersensitivity in irritable bowel syndrome. J Gastroenterol Hepatol 2011;26 Suppl 3:119-21.
22. Verdú EF, Bercik P, Verma-Gandhu M, Huang XX, Blennerhassett P, Jackson W, *et al.* Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. Gut 2006;55(2):182-90.
23. Camilleri M. Probiotics and irritable bowel syndrome: Rationale, putative mechanisms, and evidence of clinical efficacy. J Clin Gastroenterol 2006;40(3):264-9.
24. Thompson AM, Bizzarro MJ. Necrotizing enterocolitis in newbornsathogenesis, prevention and management. Drugs 2008;68(9):1227-38.
25. Panigrahi P. Necrotizing enterocolitis: A practical guide to its prevention and management. Paediatr Drugs 2006;8(3):151-65.
26. AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Evid Based Child Health 2014;9(3):584-671.
27. Demirel G, Erdeve O, Celik IH, Dilmen U. Saccharomyces boulardii for prevention of necrotizing enterocolitis in preterm infants: A randomized, controlled study. Acta Paediatr 2013;102(12):e560-5.
28. Luedtke SA, Yang JT, Wild HE. Probiotics and necrotizing enterocolitis: Finding the missing pieces of the probiotic puzzle. J Pediatr Pharmacol Ther 2012;17(4):308-28.
29. Cebra JJ. Influences of microbiota on intestinal immune system development. Am J Clin Nutr 1999;69(5):1046S-51S.
30. Gill HS, Shu Q, Lin H, Rutherfurd KJ, Cross ML. Protection against translocating *S. typhimurium* infection in mice by feeding the immuno-enhancing probiotic *L. rhamnosus* strain HN001. Med Microbiol Immunol 2001;190:97-104.
31. Haskard CA, El-Nezami HS, Kankaanpää PE, Salminen S, Ahokas JT. Surface binding of aflatoxin B(1) by lactic acid bacteria. Appl Environ Microbiol 2001;67(7):3086-91.
32. Coakley M, Ross RP, Nordgren M, Fitzgerald G, Devery R, Stanton C. Conjugated linoleic acid biosynthesis by human-derived *Bifidobacterium* species. J Appl Microbiol 2003;94(1):138-45.
33. Delia P, Sansotta G, Donato V, Messina G, Frosina P, Pergolizzi S, *et al.* Prevention of radiation-induced diarrhea with the use of VSL#3, a new high-potency probiotic preparation. Am J Gastroenterol 2002;97(8):2150-2.
34. Van Niel CW, Feudtner C, Garrison MM, Christakis DA. *Lactobacillus* therapy for acute infectious diarrhea in children: A meta-analysis. Pediatrics 2002;109(4):678-84.
35. Allen SJ, Martinez EG, Gregorio GV, Dans LF. Probiotics for treating acute infectious diarrhoea. Cochrane Database Syst Rev 2010:CD003048.
36. Isolauri E. Probiotics for infectious diarrhoea. Gut 2003;52(3):436-7.
37. Pereira DI, Gibson GR. Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. Crit Rev Biochem Mol Biol 2002;37(4):259-81.
38. Mazloom Z, Yousefinejad A, Dabbaghmanesh MH. Effect of probiotics on lipid profile, glycemic control, insulin action, oxidative stress, and inflammatory markers in patients with Type 2 diabetes: A clinical trial. Iran J Med Sci 2013;38(1):38-43.
39. Gilliland SE, Nelson CR, Maxwell C. Assimilation of cholesterol by *L. acidophilus*. Appl Environ Microbiol 1985;49(2):377-81.
40. Furrie E. Probiotics and allergy. Proc Nutr Soc 2005;64(4):465-9.
41. Yang G, Liu ZQ, Yang PC. Treatment of allergic rhinitis with probioticsn alternative approach. N Am J Med Sci 2013;5(8):465-8.
42. Giovannini M, Agostoni C, Riva E, Salvini F, Ruscitto A, Zuccotti GV, *et al.* A randomized prospective double blind controlled trial on effects of long-term consumption of fermented milk containing *Lactobacillus* casei in pre-school children with allergic asthma and/or rhinitis. Pediatr Res 2007;62(2):215-20.
43. Fond G, Boukouaci W, Chevalier G, Regnault A, Eberl G, Hamdani N, *et al.* The "psychomicrobiotic": Targeting microbiota in major psychiatric disorders: A systematic review. Pathol Biol (Paris) 2015;63(1):35-42.
44. Galland L. The gut microbiome and the brain. J Med Food 2014;17(12):1261-72.
45. Slyepchenko A, Carvalho AF, Cha DS, Kasper S, McIntyre RS. Gut emotions - mechanisms of action of probiotics as novel therapeutic targets for depression and anxiety disorders. CNS Neurol Disord Drug Targets 2014;13(10):1770-86.
46. Vitetta L, Bambling M, Alford H. The gastrointestinal tract microbiome, probiotics, and mood. Inflammopharmacology 2014;22(6):333-9.
47. Snydman DR. The safety of probiotics. Clin Infect Dis 2008;46 Suppl 2:S104-11
48. Egervärn M, Roos S, Lindmark H. Identification and characterization of antibiotic resistance genes in *Lactobacillus* reuteri and *Lactobacillus* plantarum. J Appl Microbiol 2009;107(5):1658-68.
49. Kopp MV, Hennemuth I, Heinzmann A, Urbanek R. Randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: No clinical effects of *Lactobacillus* GG supplementation. Pediatrics 2008;121(4):e850-6.
50. Sharma P, Tomar SK, Goswami P, Sangwan V, Singh R. Antibiotic resistance among commercially available probiotics. Food Res Int 2014;57:176-95.
51. Wang HF, Lin PP, Chen CH, Yeh YL, Huang CC, Huang CY, *et al.* Effects of lactic acid bacteria on cardiac apoptosis are mediated by activation of the phosphatidylinositol-3 kinase/AKT survival-signalling pathway in rats fed a high-fat diet. Int J Mol Med 2015;35(2):460-70.
52. Festi D, Schiumerini R, Eusebi LH, Marasco G, Taddia M, Colecchia A. Gut microbiota and metabolic syndrome. World J Gastroenterol 2014;20(43):16079-94.
53. de Moreno de LeBlanc A, LeBlanc JG. Effect of probiotic administration on the intestinal microbiota, current knowledge and potential applications. World J Gastroenterol 2014;20(44):16518-28.
54. Fallucca F, Porrata C, Fallucca S, Pianesi M. Influence of diet on gut microbiota, inflammation and Type 2 diabetes mellitus. First experience with macrobiotic Ma-Pi 2 diet. Diabetes Metab Res Rev 2014;30 Suppl 1:48-54.
55. Porrata-Maury C, Hernández-Triana M, Ruiz-Álvarez V, Díaz-Sánchez ME, Fallucca F, Bin W, *et al.* Ma-Pi 2 macrobiotic diet and Type 2 diabetes mellitus: Pooled analysis of short-term intervention studies. Diabetes Metab Res Rev 2014;30 Suppl 1:55-66.
56. Paolella G, Mandato C, Pierri L, Poeta M, Di Stasi M, Vajro P. Gut-liver axis and probiotics: Their role in non-alcoholic fatty liver disease. World J Gastroenterol 2014 14;20:15518-31.