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Review

Potential of probiotics as biotherapeutic agents targeting the innate immune system

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Probiotics had been of interest in the promotion of good health in animals and man. Some of the positive effects of probiotics are: growth promotion of farm animals, protection of host from intestinal infections, alleviation of lactose intolerance, relief of constipation, anticarcinogenic effect, anticholesterolaemic effects, nutrient synthesis and bioavailability, prevention of genital and urinary tract infections, and immunostimulatory effects. Their beneficial effects may be mediated by direct antagonism of specific groups of organisms, resulting in a decrease in numbers or by an effect on their metabolism or by stimulation of immunity. The resistance of people in developing countries to diseases can be improved upon by promoting the consumption of locally fermented foods that are rich in probiotic organisms. This article reviews the potential of probiotics as immuno-enhancing agents and the future perspective in developing potent probiotics with immunostimulatory molecules that can serve as outstanding vaccine adjuvants.

Key words: Probiotics, biotherapy, lactic acid bacteria, immunostimulation, CpG DNA.

INTRODUCTION

The human race and other animals have survived these many centuries because they are equipped with various defensive mechanisms (Amos, 1981). Some of these mechanisms are of general nature and serve to protect against many types of harmful agents and are on this basis referred to as non-specific immunity. A good example of this is phagocytosis of bacteria by specialised cells. Other defensive mechanisms are specific in that each is effective against a certain noxious agent and no other, and are therefore referred to as specific immunity (Stewart and Beswick, 1977). Higher animals have evolved an adaptive or acquired immune response that provides a flexible specific and more effective reaction to different infections (Roitt, 1984).

The vertebrate host does not depend solely on its immune system to protect it from the agent of disease. Other defence mechanisms, which could be mechanical such as the unbroken skin, and to a lesser extent, mucous membranes provide a physical barrier to the entrance of most pathogens. The effectiveness of these barriers is often enhanced by the presence of chemical barriers as well (Stewart and Beswick, 1977). Tears, saliva, and the secretions of the nasopharynx contain lysozome, an enzyme that digest the peptidoglycan of bacterial cell walls. Ecological controls which involve the normal body flora, exist in a balance state and thereby protect the host from invasion by pathogens (Van. der Waaij et al., 1982).

The home guard in the digestive tract are what we call ‘friendly’ bacteria. These are bacteria that fight off the potentially pathogenic ones such as *Escherichia coli* and keep our intestinal tracts ‘in balance’. When friendly bacteria are not at the appropriate levels, and when unfriendly bacteria dominate, health problems such as production of gas, bloating, intestinal toxicity, constipation, and malabsorption of nutrients can occur.

Infectious microorganisms act in certain ways that allow

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them to cause disease. These actions include gaining access to the host, adhering to and colonising cell surfaces, invading tissues, and producing toxins and other harmful metabolites (Brock and Madigan, 1991). The host however can thwart the efforts of pathogenic organisms from causing disease by their defence mechanisms. In the war of survival, if the pathogen wins, a complementary preventive and control measures will be needed.

Chemotherapeutic agent had been in use since the 1950s until its use was found to result to the development of resistant populations of bacteria, which made subsequent administration of antibiotics for therapy difficult (Fuller, 1989). The use of antibiotics as therapeutic agents had also been found to result in intestinal upsets, which often follow oral treatment with these compounds.

Innovative approaches had been tried as alternative to antibiotic and these includes using live biotherapeutic agents such as yeast (*Saccharomyces spp*.) and bacterial isolates (*Lactobacillus spp*., *Bifidobacterium* spp.) or faecal enamels (Fuller, 1992). These biotherapeutic agents are called probiotics. This review gives an overview of the beneficial effects of probiotics in animal and human health. It also look into the potential of probiotic organisms as biotherapeutic agents that can help improve host immunity to diseases through the stimulation of the innate immune system which is a highly effective set of conserved mechanisms used by multicellular organisms to recognise and counter the threat of microbial infections.

History of probitics

Microorganisms have been essential to food and alcohol fermentation for thousands of years. Over the last century, different microorganisms have been used for their supposed ability to prevent and cure diseases, leading to the coining of the term probiotics, or 'pro-life' (Lilly and Stillwell, 1965). The concept of probiotics evolved around 1900. At this time Henry Tissier, a French Paediatrician, observed that children with diarrhoea had in their stools a low number of bacteria characterised by a peculiar, Y shaped morphology. These "bifid" bacteria were, on the contrary, abundant in healthy children (Tissier, 1906). Nobel price-winning Elie Metchnikoff in 1907 advocated that the consumption of lactobacilli helps in controlling endogenous intoxication (autointoxication) caused by wrong types of components in the intestinal flora. He pointed out that the long, healthy lives of Bulgarian peasants were the result of their consumption of fermented milk products. The works of Metchnikoff and Tissier were the first to make scientific suggestions about the probiotic use of bacteria. The first clinical trials were done in the 1930s on the effect of probiotics on constipation (Koop-Hoolihan, 2001).

A lot of research on probiotics had been carried out after that time and it is increasing steadily since then, but much of it is in Europe, Asia, and America, and of recent in South Africa. Presently, probiotics are available in a variety of food products and supplements. In the U.S.A., the food products containing probiotics are almost exclusively dairy products, fluid milk and yoghurt, due to the historical association of lactic acid bacteria with fermented milk (Koop-Hoolihan, 2001). The most frequently used bacteria in these products belong to the genera *Lactobacillus,* Bifidobacterium, and *Streptococcus.*

There have been several definitions to the word probiotics over the years. Lilly and Stillwell in 1965 used it to describe substances produced by one protozoan which stimulates another, but Parker in 1974 described it as animal feed supplements which had a beneficial effect on the host animal by affecting its gut flora. Parker's definition clearly mentions organisms and substances, which contribute to intestinal microbial balance. Fuller (1989) argued that the latter definition is too imprecise, since substances mentioned would include antibiotics. He later revised the definition as 'a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance'. This new definition emphasises the importance of live cells as an essential component of an effective probiotic and removes the confusion created by the use of the word substances. Other workers had also given their own definition of the term probiotic. For instance, Donohue et al. (1998) described probiotic bacteria as viable bacteria when applied in single or mixed culture, exhibit a beneficial effect on the health of the host. This definition encompasses the application of either axenic or mixed culture in the treatment of disease.

The most recent definition was by Schrezenmeir and De Vrese (2001). They defined probiotics as viable microbial food supplements which beneficially influence the health of the host. This new definition clearly points out the health promoting effect of probiotic agents. For clarity purpose, the following terms can be used to distinguish different probiotic microorganisms:

Research strain: This is any generally regarded as safe (GRAS) microorganism being studied for probiotic application, but not commercially available in any market.

Commercial strain: A strain produced on an industrial scale for commercial use, as a fresh product (fermented milk, juice etc) or nutritional supplement (capsules or sashes).

Probiotic strain: Any generally regarded as safe (GRAS) microorganism (such as *lactobacilli*, bifidobacteria, *streptococci, saccharomyces,* etc) shown in published research to have one or more of the following positive attributes:

- *In vitro* adherence to epithelial cells.
- *In vitro* antimicrobial activity
- *In vitro* resistance to bile, hydrochloric acid, and pancreatic juice.
- Anticarcinogenic activity (reduction of carcinogens) in clinical trials.
- Immune modulation or stimulation in clinical trials.
- Reduction of intestinal permeability in clinical trials.
- Colonisation of the GIT in clinical trials.

Implantable strain: Any microbial strain native to the GIT of man (that is, *lactobacilli* or bifidobacteria) shown to survive passage through the GIT (appear live in stool) or persist on biopsies of the GIT mucosa after cessation of feeding.

Clinical strain: An implantable strain which has been shown to have one or more specific health benefits, and therefore have demonstrated clinical usefulness. Some examples of benefits that have been shown are reduced intestinal permeability, enhancement of immune functions, and treatment of infection.

Composition of probiotics

Probiotics can be compounded in various ways depending on the sort of use intended. They can either be included in the pelleted feed or produced in the form of capsules, paste, powder or granules which can be used for dosing animals directly or through their food (Fuller, 1989). Probiotic preparations may be made up of a single strain or may contain any number up to eight strains. The advantage of multiple strain preparations is that they are active against a wide range of conditions and in a wider range of animal species.

Fuller (1989) listed the following organisms as species used in probiotic preparation: *Lactobacillus bulgaricus, Lactobacillus plantarum, Streptococcus thermophillus, Enterococcus faecium, Enterococcus faecalis, Bifidobacterium* species, and *Escherichia coli*. With the exception of *L. bulgaricus* and *S. Thermophilus*, all the other organisms are all intestinal strains.

Lactobacilli, Streptococci and Bifidobacteria are the commonly used groups in the production of probiotics. The justification for the use of *Lactobacilli* stems from studies which show that when the gut flora develops after birth, as *Lactobacilli* increases, other components of the flora decrease (Smith, 1965). Fuller (1978) had also reported that *Lactobacilli* exert a controlling effect on *E. coli* in gnotobiotic chicks.

Mode of action of probiotics

The mechanisms by which probiotics exert their effects on the host are still speculative (Koop-Hoolihan, 2001). Their beneficial effects may be mediated by direct antagonistic effect against specific groups of organisms, resulting in a decrease in numbers or by an effect on their metabolism or by stimulation of immunity. Probiotics antagonise pathogens through production of antimicrobial and antibacterial compounds such as cytokines and butyric acid (DeVuyst and Vandamme, 1994; Kailasapathy and Chin, 2000); reduce gut pH by stimulating the lactic acid producing microflora (Langhendries et al. 1995); compete for binding and receptor sites that pathogens occupy (Fujiwara, 1997; Kailasapathy and Chin, 2000); improve immune function and stimulate immunomodulatory cells (Isolauri et al., 1995; Rolfe, 2000); compete with pathogens for available nutrients and other growth factors (Rolfe, 2000); or produce lactase which aids in lactose digestion.

Characteristics of good probiotics

Fuller (1989) listed the following as features of a good probiotic:

- It should be a strain, which is capable of exerting a beneficial effect on the host animal, e.g. increased growth or resistance to disease.
- It should be non-pathogenic and non-toxic.
- It should be present as viable cells, preferably in large numbers.
- It should be capable of surviving and metabolising in the gut environment e.g. resistance to low pH and organic acids.
- It should be stable and capable of remaining viable for periods under storage and field conditions.

A probiotic agent with all these features has considerable advantage over antibacterial supplements such as antibiotics currently in use. They do not induce resistance to antibiotics, which will compromise therapy. They are not toxic and therefore will not produce undesirable side effects when being fed and in the case of food animals, will not produce toxic residues in the carcass. They may stimulate immunity whereas the immune status remains unaffected by antibiotics.

An essential determinant in the choice of a probiotic microorganism is its ability to reach, survive, and persist in the environment in which it is intended to act (Marteau et al., 1992; Charles et al., 1998).

Nutritional and health promoting effects of probiotics

Modern nutrition is changing rapidly. In recent time, the focus of scientist has been to look into the health promoting effect of food apart from safe nutrient provision and overcoming deficiencies. It is anticipated that this kind of food called functional foods, will contribute to an overall better state of health for the consumers. Probiotic preparations have served as functional foods in human and animal production for the past two decades. The beneficial effects of probiotic will depend on a number of factors including the strain chosen, level of consumption, duration and frequency of exposure, and the physiological condition of the individual (Koop-Hoolihan, 2001). Some of the beneficial effects of the practical use of probiotics are: growth promotion of farm animals (Baird, 1977; Mordenti, 1986; Chang et al., 2001); protection of host from intestinal infections (Nurmi and Rantala, 1973; Pascual et al., 1999; Koop-Hoolihan, 2001; Oyetayo et al., 2003); alleviation of lactose intolerance (Garvie et al., 1984; Jiang, 1996); relief of constipation (Alm et al., 1983; Graf, 1983); anticarcinogenic effect (Fuller, 1989; Walker and Duffy, 1998; Zabala et al., 2001); anticholesterolaemic effects (Tahri et al., 1995; Bertazzoni et al., 2001); nutrient synthesis and bioavailability (Koop-Hoolihan, 2001); prevention of genital and urinary tract infections (Redondo-Lopez et al., 1990; Martin et al., 1999); immunostimulatory effects (Aattouri et al., 2001).

Effects of probiotics on the immune system

The focus of the present review is on the potentials of probiotics to stimulate the immune system of the host. It has been discovered that conventional animals with a complete gut flora have increased phagocytic activity and immunoglobulin levels compared with germ-free animals (Baalmear et al., 1984). Yoghurt has been shown to increase antibody levels when fed to germ free mice (Wade et al., 1984). Lactobacilli casei in particular was found to be active in the stimulation of phagocytic activity when administered to mice (Perdigon et al., 1986). For bacteria to be effective in the process of immunostimulation, it may be necessary for them to migrate from the gut to the systemic circulation. Lactobacilli had been found to be capable of translocating and surviving for many days in the spleen, liver, and lungs (Fuller, 1989).

Immune modulation of blood leukocytes in humans by lactic acid bacteria (LAB) had also been observed (Schiffrin et al., 1997). Aattouri et al. (2001) reported that oral ingestion of LAB by rats increases lymphocyte proliferation and interferon production. They suggested that beneficial consequences might be obtained by an increase in resistance to some infections.

Studies in rats and mice reveal that lactic acid bacteria administered orally increase the numbers of T lymphocytes, $CD4^+$ cells and antibody-secreting cells, including those in the intestinal mucosa, and enhance lymphocyte proliferation, natural killer cell activity, IL-1, TNF and IFN-γ production, antibody production (including secretory IgA), phagocytic activity and the respiratory burst of macrophages and the DTH response (Naidu et al., 1999). It has also been reported that not all strains of lactic acid bacteria are effective (Naidu et al., 1999). In a study on rats co-colonised with *L. plantarum* and *E. coli,* Herias et al. (1999) reported a higher circulating concentration of total IgA and of *E. coli*-specific IgA and IgM compared with rats which were colonised with *E. coli* alone. There was also increased expression of the IL-2 receptor in the lamina propria. Though there had been several animal studies, the effects of probiotic bacteria on human immune function are still controversial (Naidu et al., 1999).

Conclusion and future research perspective

The potential of probionts associated with fermented African foods and beverages, such as ogi and kunnu, to modulate host immunity is a very promising area of research. More emphasis should be on further screening of already discovered probiotics e.g *L.plantarum* isolated from ogi slurry (Oyetayo and Osho, 2004) and also new probiotic candidates from other sources. This will involve further *in vitro* and *in vivo* investigation to evaluate their potential as immunostimulatory agents. Furthermore, genetic engineering of already identified probiotics and those newly discovered to make them more efficacious should be pursued.

Further studies should be focused on the mechanisms of action within system, which stimulate the *in vivo* effects. An understanding of the oligonucleotide sequence, which triggers innate immune system, will go a long way to help understand the mechanism by which immunostimulatory potentials of probiotics are mediated. This investigation will reveal which CpG motifs within probiotic DNA (CpG DNA) that is actually responsible for the stimulation of the immune system of humans. An understanding of the specific oligonucleotide sequence will help in developing synthetic oligonucleotides containing CpG motifs (CpG DNA), which are potent immunostimulatory molecules and outstanding vaccine adjuvants. This can be achieved by genetic modification of these probionts associated with fermented foods and beverages indigenous to Africa.

REFERENCES

Aattouri N, Bouras M, Tome D, Marcos A, Lemonnier D (2001). Oral ingestion of lactic acid bacteria by rats increases lymphocyte proliferation and interferon -γ production. British J. 87: 367–373.

Alm L, Ryd-Klellen E, Setterberg G, Blomquist I (1993). Effect of a new fermented milk product ‘cultura’ on constipation in geriatric patients. In: The lactic acid bacteria. Pro. 1st lactic acid bacteria computer conference, Eds. EL Foo, HG Griffin, R Mollby, CG Heden. Horizon sci. press, Norfolk, Engl. pp. 13–17.

Amos WMG (1981). Basic immunol. Butterworths and Co. pp. 115–121.

Baalmear PM, Holtermann OA, Mirand EA (1984). Influence of the microflora on the immune response 1: General characteristics of the germ free animal. In the germ-free animal in biomedical research. Eds. Coates, M.E. and Gustafsson, BE, pp. 335–346. Lond. Lab. Anim. Ltd.

Baird DM (1977). Probiotics help boost feed efficiency. Feed stuffs.49: 11–12.
Bertazzoni ME, Benini A, Marzotto M, Hendriks H, Sbarbati A, Dellaglio F (2001). Preliminary screening of the health promoting properties of new lactobacilli strains: *in-vitro* and *in-vivo*. HEALFO conference, Santa Maria Imbaro and Lanciano - Italy. 13–15 June.
Brock TD, Madigan MT (1991). Host-Microbe relationships and disease processes. In Biology of Microorganisms 6th edition. Prentice-Hall Inc. pp. 379–380.
Chang Y, Kim J, Kim W, Kim Y, Park Y (2001). Selection of a potential probiotic lactobacillus strain and subsequent *in-vivo* studies. Antonie Van Leeuwenhoek. pp. 193–199.
Charles WP, Kelly PM, Morelli L, Collins JK (1998). Antibiotic Susceptibility of potentially probiotic *Lactobacillus spp.* J. food protect. 61: 1636–1643.
DeVuyst L, Vandamme EL (1994). Antimicrobial potential of lactic acid bacteria. In bacteriocins of lactic acid bacteria. Ed. DeVuyst, L. and Vandamme, EL London: Blackie acad.and professional. pp. 91–142.
Donohue DC, Salminen S, Marteau P (1998). Safety of probiotic bacteria. In lactic acid bacteria eds. Salminen S, von Wright A. New York: Marcel Dekker. pp. 369–383.
Fujiwara S, Hashiba H, Hirota T, Forstner JF (1997). Proteinaceous factor(s) in culture supernatant fluids of bifidobacteria which prevents the binding of enterotoxigenic *Escherichia coli* to gangliotetraosylceramide. Appl. Environ. Microbiol. 63:506–512.
Fuller R (1978). Epithelial attachment and other factors controlling the colonisation of the intestine of the gnotobiotic chicken by lactobacilli. J. Appl. Bacteriol. 46:335–342.
Fuller R (1989). Probiotics in man and animals – A rev. J. of Appl. Bacteriol. 66:365–378.
Fuller R (1992). Probiotics: The scientific basis. Ed. Fuller R. London: Chapman and Hall.
Garvie EI, Cole CB, Fuller R, Hewitt D (1984). The effect of yoghurt on some components of the gut microflora and the metabolism of lactose in the rat. J. Appl. Microbiol. 56:237–245.
Graf W (1983). Studies on the therapeutic properties of acidophilus milk. Symposia of Swedish Nutrition Foundation XV, 119–121.
Herias MV, Hessle C, Telemo E, Midtvert T, Hanson LA, Wold AE (1999). Immunomodulatory effects of *Lactobacillus plantarum* colonising the intestine of gnotobiotic rats. Clinical and Exp. Immunol. 116: 283–290.
Isolauri E, Joensuu J, Suomalainen H, Luomala M, Vesikari T, (1995). Improved immunogenicity of oral DSRRV reassortant rotavirus vaccine by *Lactobacillus casei GG*. Vaccine 13: 310–312.
Jiang T, Mustapha A, Savaiano DA (1996). Improvement of lactose digestion in humans by ingestion of unfermented milk containing *Bifidobacterium longum.* J. Dairy sci. 79 (5): 750–757.
Kailasapathy K, Chin J (2000). Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium spp.* Immunol. and cell Biol. 78 (1): 80–88.
Koop-Hoolihan L (2001). Prophylactic and therapeutic uses of probiotics: A rev. J. of the Am. Dietetic Assoc. File: //G:/ Intern. 1.
Langhendries JP, Detry J, Van Hees J, Lamboray JM, Darimont J, Mozin MJ, Secretin MC, Senterre J (1995). Effect of a fermented infant formula containing viable bifidobacteria on the faecal flora composition and pH of healthy full-term infants. J. pediatrics gastroenterology nutr. 21: 177–181.
Lilly DM, Stillwell RH (1965). Probiotics: Growth promoting factors produced by microorganisms. Sci.147: 747–748.
Marteau P, Pochart P, Bounik Y, Zidi S, Goderel J, Rambaud JC (1992). Survival of *Lactobacillus acidophilus* and *Bifidobacteria* spp.
ingested in fermented milk in the small intestine: A rational basis for the probiotics in man. Gastroenterologie clinique et biologue. 16: 25–28.
Martin HL, Richardson BA, Nyange PM, Lavreys L, Hillier SL, Chohan B, Mandaliya K, Ndinya-Achola JO, Bwayo J, Kreiss J (1999). Vaginal lactobacilli microbial flora and risk of human immunodeficiency virus Type 1 and sexually transmitted disease acquisition. J. Infectious disease 180: 1863–1868.
Metchnikoff E (1907). The prolongation of life. Optimistic studies. G.P. Putman,s sons. Mitsuoka T (1992). The human gastrointestinal tract. In the lactic acid bacteria in health and disease Vol. 1. ed. Wood B.J.B. Elsevier appl. Sci. Lond. pp. 69–114.
Mordenti A (1986). Probiotics and new aspects of growth promoters in pig production. Information Zootechnol. 32: 69.
Naidu AS, Bildlack WR, Clemens RA (1999). Probiotic spectra of lactic acid bacteria (LAB). Critical rev. in food Sci. nutr. 39 (1): 1–26.
Nurmi IE, Rantala M (1973). New aspects of salmonella infection in broiler production. Nat. 24: 210–211.
Oyetayo VO, Adetuyi FC, Akinyosoye FA (2003). Safety and protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei* used as probiotic agent *in vivo.* Afr. J. Biotechnol. Vol. 2 (11) pp. 448–452.
Oyetayo VO, Osho B (2004). Assessment of probiotic properties of a strain of *Lactobacillus plantarum* isolated from fermenting corn slurry. J. of Food, Agric. Environ. Vol. 2 (1): 132–134.
Pascual M, Hugas M, Badiola JI, Monfort JM, Garriga M (1999). *Lactobacillus salivarius* CTC 2197 prevents *Salmonella enteritidis* colonisation in chickens. Appl. Environ. Microbiol. 65:4981–4986.
Perdigon G, Macais MEN, Alvarez S, Oliver G, De Ruiz-Holgado AAP (1986). Effect of perorally administered lactobacilli on macrophage activation in mice. Infection and Immunity 53:404–410.
Rendondo-Lopez V, Cook RL, Sobel JD (1990). Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. Rev. infectious Diseases 12:856–872.
Rolfe RD (2000). The role of probiotic cultures in the control of gastrointestinal health. J. Nutr. 130 (2S):396S–402S.
Roitt I (1984). Essential Immunology, 5th ed.Blackwell Scientific Publications, pp. 1- 7.
Schiffrin EJ, Brassart D, Servin AL, Rochat F, Donnet-Hughes A (1997). Immune modulation of blood leucocytes in humans by lactic acid bacteria: Criteria for strain selection. Am. J. Clinical Nutr. 66 (2):515S–520S.
Schrezenmeir J, de Vrese M (2001). Probiotics, prebiotics and synbiotics: approaching a definition. Am. J. Clinical Nutr. 73 (2):361S–364S.
Smith HW (1965). Observations on the flora of the alimentary tract of animals and factors affecting its composition. J. Pathol. Bacteriol. 89:95.
Stewart FS, Beswick TSL (1977). Bacteriology, virology and immunity for students of medicine. 10th edn. Macmillan publishing Co. Inc., New York. pp. 47–58.
Tahri K, Crociani J, Ballongue J, Schneider F (1995). Effects of three strains of bifidobacteria on cholesterol. Letl. Appl. Microbiol. 21 (3): 149–151.
Tissier H (1906). Traitement des infections intestinales par la methode de la flore bacterienne de l'intestin. CR. Soc. Biol. 60:359–361.
Vander Waaij D, Horstra H, Wiegersma N (1982). Effect of ß-lactam antibiotics on the resistance of the digestive tract to colonisation. J. infectious Diseases 146:417–422.
Wade S, Corthier G, Moreau L, Besnier MO (1984). L'ingestion de yaout vivant modifie-t-elle la response immunitaires? IDF Bull. N0. 179. p. 1.
Walker AW, Duffy LC (1998). Diet and bacterial colonisation: Role of probiotics and prebiotics: Rev. J. Nutr. Biochem. 9: 668–675.
Zabala A, Martin MR, Haza AI, Fernandez L, Rodriguez JM, Morales P (2001). Anti-proliferative effect of two lactic acid bacteria strains of human origin on the growth of a myeloma cell line. Letl. Appl. Microbiol. 32: 287–292.