Probiotic Association of India

From The Chief Editor's Desk

Dear Members/Viewers,

Season's choicest greetings from PAi Newsletter team to all of you!

As you all know, probiotics continue to be in the lime light both in India and other parts across the world inspite of the stringent stand taken by International regulatory authorities like EFSA from health claim perspectives. There continues to be a boom in world market. Thanks to the tremendous enthusiasm and confidence shown by the consumers towards probiotics and their formulations as an effective dietary based strategy to manage their health and well being. This has largely been possible due to the joint efforts of all the stake holders starting with high quality scientific publications coming out from the laboratories of various research groups in the country and rest of the world, food and pharma industry and organization of scientific programmes by various National and International associations for the promotion of probiotic products amongst the native populations. Last but not the least, our Probiotic Newsletter have also turned out to be a strong linkage with the society to apprise the consumers and other stake holders about the new developments, launch of new probiotics strains and their formulations in the market. I'm sure; all of you must be anxiously waiting for the launch of the 8th issue of PAi newsletter since the date for the same been fast approaching. I am sure you will not be disappointed.

It gives me immense pleasure to inform you that the 8th issue of PAi Probiotic Newsletter has been launched and will be uploaded on PAi website soon after being sent to all the members. Hope you will find it interesting. We would appreciate receiving your comments on the quality of the information figuring in the issue alongwith valuable inputs for further improvements with an open mind. It will be a great favor to us if you could help us in giving a new shape to the Newsletter by further broadening the scope of Probiotic concept by value addition beyond the food and pharma formulations already available in the market .

Dr J.B. Prajapati received Hari Ohm Ashram Award from ICAR

Dr Jashbhai B Prajapati, Professor & Head, Department of Dairy Microbiology, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat has been conferred coveted Hari Ohm Ashram Award for Biennium 2012-13 (Animal and Fisheries Sciences) by Indian Council of Agricultural Research, New Delhi. This award was given in recognition of his outstanding contributions in the area of probiotic research. The award carries a citation and a cash prize of Rs. One lakh which was given in ICAR award ceremony at Patna on 25th July, 2015.

Dr J. B. Prajapati organizes a session on Probiotics at Food Technologists Conference IFT15

Dr J.B. Prajapati, Professor and Head of Dairy Microbiology Department at SMC College of Dairy Science, Anand Agricultural University, Anand participated in Institute of Food Technologists conference IFT15 at Chicago, USA during 9-14 July, 2015. He along with some of his past students organized a session on "Adding value to food and nutrition through probiotics : Present status and future perspective". Among the three speakers in the session, Dr Suja Senan (South Dakota State University) talked on Role of probiotics and controlling obesity and weight management: A protein-probiotic interaction and Dr James Steele (University of Wisconsin) presented on use of genomics in probiotic research. Dr Prajapati gave lead talk on "Role of probiotics in gut health- Past, Present and Future". He conversed about the concept of consumption of fermented milk, which centered on beneficial bacteria and their health effects in the gut to establish that the association of man and microbes was known since ancient times. He explained the importance of microbes in gut as they offer protective, metabolic and trophic functions. Hence, modulation of gut flora with beneficial bugs - probiotics will positively affect all these functions. Present knowledge about health effects of probiotics in different formats was presented and future perspectives on novel probiotics, their role in prevention and control of life style diseases, metagenomics and nutrigenomics were also dealt with by Dr Prajapati.

Two Posters entitled "Development and evaluation of probiotic starter culture dosage forms" by J.B. Prajapati and Sreeja Mudgal and "Comparison of traditional plating and real time PCR-Based quantification of probiotic strains from human fecal samples after probiotic therapy" by Suja Senan, J. B. Prajapati, C.G. Joshi and H. A. Patel were also presented.

Dr J. B. Prajapati at Yakult symposium

Dr J B Prajapati was invited to talk on "Probitoics in geriatric population" at "Probiotics - from Bench to Community" a symposium organized by Yakult India Microbiota and Probiotic Science Foundation during 7th - 8th March, 2015 at New Delhi. He presented results of a clinical trial conducted to study the effect of probiotic intervention on immunological and microbiological functions in geriatric population.

Bifidobacteria Probiotic as a preventive measure for rheumatoid arthritis: a hypothesis

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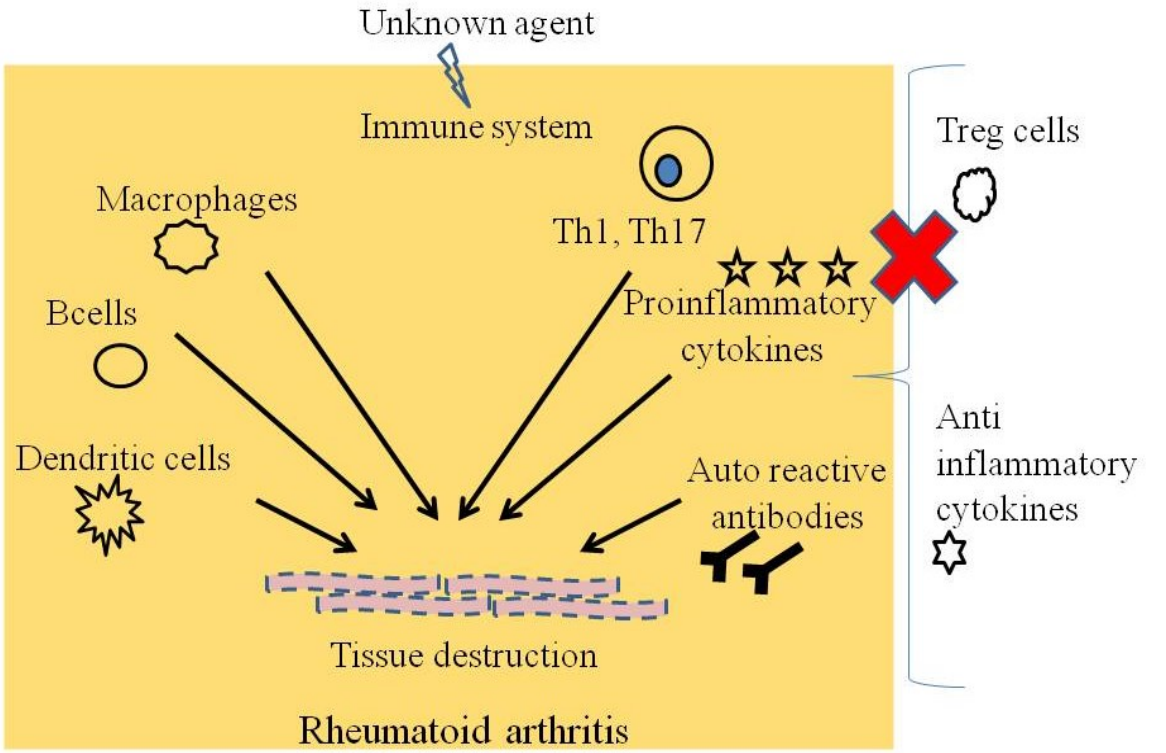
The concept of modulation of gut microbiota with health promoting microbes introduced by Elie Metchnikoff has been the essence of probiotics. Initial studies of probiotics were much focussed on localised gastrointestinal infections. However with current reports of association of gut microbiota with development of immune system, metabolic disorders and influence on brain, probiotics are foreseen as a remedy for non-localised diseases.

Bifidobacteria, one of the dominant gut microflora, has been identified to play an important role in development of immune system. The role of bifidobacteria in maintenance of tolerance can be exploited in cases where tolerance system fails resulting in immunological disorders. Rheumatoid arthritis (RA) is one such autoimmune disorder primarily affecting the synovial tissue followed by cartilage and bone destruction. Though the exact aetiology of RA remains to be identified, it is generally considered to be failure of immune regulating system. RA is featured by presence of auto-reactive antibodies, cytokines, macrophages, dendritic cells, T cells and B cells which are involved in tissue destruction.

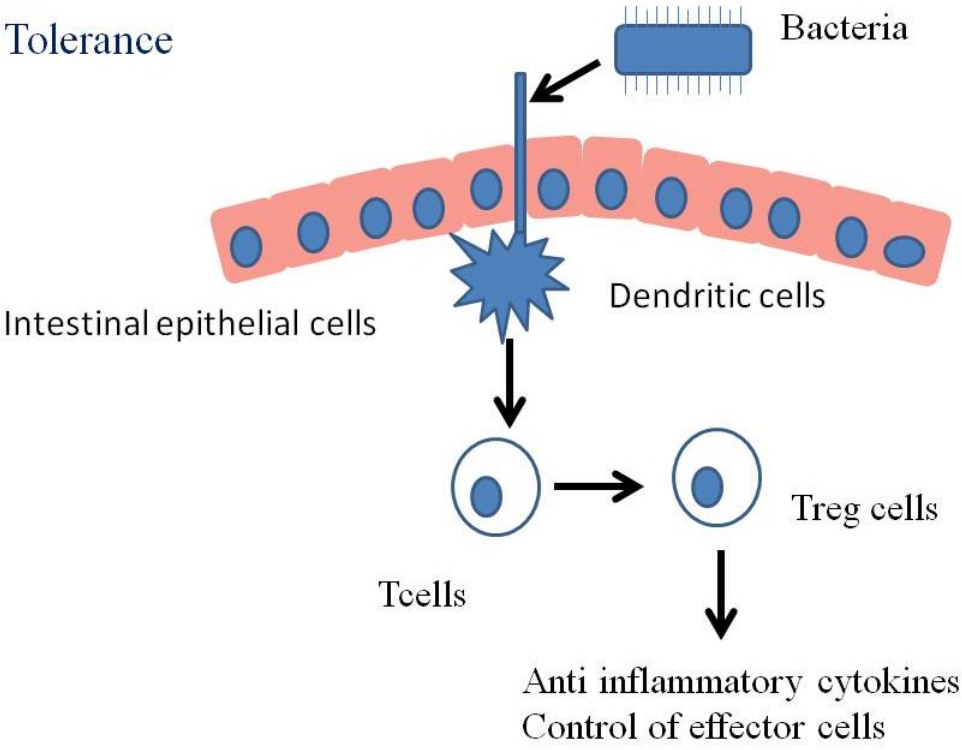
Several subsets of T cells are suggested to have a role in the pathogenesis of RA. However, reports indicate influence of Th1 and Th17 cytokines in the progression of disease; further a disturbed Treg cell functioning is also accused for the development of RA. Though the exact functions of Treg cells are not clear, they are supposed to be involved in suppressing the effector T cell response. Pro inflammatory cytokines such as IL1, TNF α, IL6, IL15, IL 17, IL18, GM CSF etc. are held responsible for synovial inflammation. In addition, the levels of anti-inflammatory cytokines IL10, IL1Ra are present in insufficient amounts to counteract pro-inflammatory cytokines as shown in following figure.



An unknown agent triggers immune system which leads to uncontrolled effector cell response (shaded region), there is lack of sufficient anti-inflammatory cytokines and Treg cells to control the reaction which result in damage of tissue

Bifidobacterial crosstalk with intestinal epithelial cells can result in release of chemokines and cytokines which can direct trafficking and recruitment of immune cells. Recent literature document the ability of bifidobacteria to induce Treg cells (Konieczna et al., 2012) and these cells can control responses to self and intraluminal antigens by curbing uncontrolled effector response. Many *in vitro* studies have demonstrated the ability of bifidobacterial strains to activate anti-inflammatory cytokines and reduce the induction of pro-inflammatory cytokines in response to pathogen. Bifidobacteria has also been reported for attenuation of inflammation following a single ovalbumin challenge in mice model for allergen induced lung inflammation. (MacSharry et al., 2012).

In case of rheumatoid arthritis the proficiency of bifidobacteria to generate such systemic effect has to be examined. *L. casei* has been shown to reduce levels of reactive pro-inflammatory cytokines in case of collagen induced arthritis in female *Lewis* rats by down regulating Th1 effector functions (So et al., 2008). In another study, *L. casei* and *L. acidophilus* have been shown to reduce oxidative stress and pro-inflammatory cytokines in collagen induced *Wistar* rats (Amdekar et al., 2013). Studies reveal that Bifidobacterial levels in case of rheumatoid arthritis patients are low (Vaahtovuo et al., 2008). Hence, the influence of bifidobacteria on arthritis needs to be investigated and as a result prevention of progression of disease by restoration of bifidobacteria levels through a probiotic has to be evaluated.



Probiotic bacteria are involved in cross talk with gut epithelial cells, dendritic cells recognises these bacteria and present antigen to naive Tcells which drive their differentiation to Treg cells. These Treg cells promote tolerance by release of anti-inflammatory cytokines and control of effector cells

References:

Amdekar S, Singh V, Kumar A, Sharma P, Singh R (2013) *Lactobacillus casei* and *Lactobacillus acidophilus* regulate inflammatory pathway and improve antioxidant status in collagen-induced arthritic rats. *J Interferon Cytokine Res.* 33(1):1-8

Konieczna P, Groeger D, Ziegler M, Frei R, Ferstl R, Shanahan F, Quigley EM, Kiely B, Akdis CA, O'Mahony L. (2012) *Bifidobacterium infantis 35624* administration induces Foxp3 T regulatory cells in human peripheral blood: potential role for myeloid and plasmacytoid dendritic cells. *Gut*. 61(3): 354-66

MacSharry J, O'Mahony C, Shalaby KH, Sheil B, Karmouty-Quintana H, Shanahan F, Martin JG.(2012) Immunomodulatory effects of feeding with *Bifidobacterium longum* on allergen-induced lung inflammation in the mouse. *Pulm Pharmacol Ther*. 25(4):325-34

So JS, Kwon HK, Lee CG, Yi HJ, Park JA, Lim SY, Hwang KC, Jeon YH, Im SH. (2008) *Lactobacillus casei* suppresses experimental arthritis by down-regulating T helper 1 effector functions. *Mol Immunol.* 45(9):2690-9.

Vaahtovuo J, Munukka E, Korkeamäki M, Luukkainen R, Toivanen P (2008) Fecal Microbiota in Early Rheumatoid Arthritis. *J Rheumatol* 35 (8):1500-5.

Probiotic Soy Yoghurt and Health Potential

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Soybean is economically the most important bean in the world, providing high quality vegetable protein for millions of people and ingredients for hundreds of chemical products as well as potential source of bioactive peptides. Soybean also contains carbohydrates, phytochemicals, saponins, phytic acid and fiber, all thought to provide health benefits. Phytochemicals include the isoflavones (genistein, daidzein and glycitein), phytic acid and saponins. Soybeans are converted into different food products such as soymilk, tofu, soy protein isolates, soy flour based products etc. However, consumption of soymilk is undesirable due to the presence of unpleasant off-flavors (beany) carried over from soybeans. Moreover, soymilk contains flatulence causing oligosaccharides including raffinose and stachyose. The problems in soymilk can be resolved partly by lactic fermentation, and hence production of fermented soymilks such as soymilk yogurt is important. Soy foods may serve as the ideal systems for the delivery of probiotic bacteria to the human gastrointestinal tract (GIT), since they may provide a favourable environment, which promotes growth and enhances viability of these microorganisms. In dairy and soy applications, probiotic organisms are delivered with different fermented dairy and soy products, most notable are yoghurt and soy yoghurt.

Soy yoghurt is fermented soymilk made with a mixed starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Incorporation of probiotic organisms such as *Lactobacillus acidophilus, Bifidobacterium* sp. and *L. casei* in fermented products provides a potential to improve the quality of the product and the health status of consumers.

During the bacterial fermentation, major constituents including soy proteins, raffinose, stachyose and other soy carbohydrates are utilized for the bacterial growth, which results in the conversion of fermentable materials into a range of products such as lactic acid, acetic acid, peptides, amino acids and different vitamins. Isoflavones have been found to increase the activities of some antioxidative enzymes in the liver. Some of these bioactive compounds are considered functional, thus making dairy and soy products important part of functional foods and nutraceuticals. In case of fermented soy product, the developed constituents (lactic acid and acetic acid) and probiotics can influence the absorption or metabolism of isoflavones.

Probiotics- the multifunctional magical bowl for the healthy gut

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The research into the health benefits of probiotics and prebiotics has rocketed sky high over the past few decades. There are some of the novel applications with regard to diseases and disorders for these healthy dietary components that were previously unimaginable. New applications of live bacteria in food services probiotics and protective cultures have become time-honored. Probiotic organisms are evident to offer several functional properties including stimulation of immune system. Probiotics have many nutritional and therapeutic benefits as they are used in the prevention and treatment of Inflammatory Bowel Diseases (IBD). Recent studies in mice provided evidence that intestinal microflora that have been genetically modified to secrete appetite suppressing metabolites could help against obesity and type 2 diabetes (Yadav *et al*., 2013).

The intestinal microbiota forms a complex ecosystem that has an important impact on the health of a host, and an increasing number of disorders are associated with disturbances in this ecosystem. There is budding evidence that even brain function can be affected by an anomalous gut microbiota and that the bidirectional signaling. The microbe-gut-brain axis also plays a significant role in the well-being of the gut as well as the brain. In addition, its role is to explicate the link with disorders such as anxiety, depression, autism spectrum disorder and possible beneficial action of prebiotics and probiotics.

Some of the probiotic bacteria such *Lactobacillus reuteri* biofilms produce antimicrobial and anti-inflammatory factors and it also forms biofilms. Probiotics have a key role in aiding to maintain hormonal balance in women of all ages, from the menstruating years all the way through to the post-menopausal years.

Probiotics have been widely used in livestock rearing such as aquaculture, to elevate the production. Probiotics can also improve water quality and pond management. Research in probiotics for aquaculture such as finfish and shell fish culture is at an early stage of development and much work is still needed. Since the development of antibiotic resistance has become a theme of growing concern, there is an essential need in aquaculture to develop microbial control strategies, since disease outbreaks are recognized as important constraints to aquaculture production and trade. One of the alternatives to anti-microbials in disease control could be the use of probiotic bacteria as microbial control agents.

Reference :

1. Yadav, H., Lee, Ji-Hyeon , Lloyd, J., Walter, P. and Rane, S. G. (2013). Beneficial Metabolic Effects of a Probiotic via Butyrate-induced GLP-1 Hormone Secretion. ***J. Biol. Chem***.288(35):25088-25097.

Cost effective food grade medium for *Lactobacillus* sp.

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Large quantity of whey is generated as by-product from paneer, cheese and casein industries. Much of this whey remains unutilized and goes as waste, and thus, a huge amount of valuable milk nutrients (Protein, NPN, lactose, vitamins, calcium, phosphorus, magnesium, potassium, etc.) are lost. The high level of lactose (4–5%) in whey, mainly responsible for this high BOD value (0.65 g/ g lactose), promotes the multiplication of lactic acid bacteria (LAB). Their complex nutrient requirements are usually satisfied by natural sources of synthetic growth media, containing matrices of undefined and complex composition, such as yeast extract and peptones of various origins. An appropriate low cost, food grade medium is essential for mass production and preservation of lactobacilli for food application. Semi-synthetic liquid medium, skim milk, butter milk and cheese whey-based media are commonly used for starter culture production. Whey-based medium requires additional nutrients supplementation for the maximum growth of lactobacilli. A cost effective food grade medium for *Lactobacillus* sp. for culturing and biomass production using of whey has been formulated. Growth performances of *Lactobacillus* sp. in formulated whey based media are equivalent to laboratory media used for culturing of *Lactobacillus* sp. Cost of medium is less than the commercially available media. Medium is suitable for production of probiotic importance *Lactobacillus* sp. biomass at large scale.

Direct Product Probiotic (DPP) formulation of *Lactobacillus* culture

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Some strains of the genus *Lactobacillus* have attracted great attention due to beneficial effects to human and have been attributed as “Probiotics”. Numerous fermented milk products containing probiotic lactobacilli have recently been commercialized throughout the world. Certain probiotic lactobacilli grow slowly in milk. Therefore, some products are produced by co-culture with ordinary starter bacteria after the addition of sufficient amounts of separately propagated viable probiotic cells. A bioprocess for production of *Lactobacillus* sp. biomass, harvesting and preservation in dried form has been developed. Processes for production of *Lactobacillus* sp. biomass under batch and fed batch scale fermentation have been optimized. Protocol for harvesting of cell biomass from cultured medium has been standardized by microfiltration and centrifugation for efficient recovery. Preservation of cell biomass as freeze dried powder has been optimized. Viable counts in dried preparation were 11 -12 log cfu/g, which was stable till 75 days at -20°C. Concentrated and stable *Lactobacillus* culture can be used as DPP in functional food formulations and supplements.

Acid and bile tolerance attributes of Lactobacilli of food and faecal origin

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Lactobacilli are major part of the probiotic fermented dairy products, significant microflora of the intestine and are also found in a variety of environments. Before considering an organism as a probiotic strain, some desirable in vitro functional attributes serve as important criteria for their selection. The aim of this study was to find out the ability of acid and bile tolerance properties of food and faecal origin lactobacilli. In the present study, the most prominent twenty isolates were identified as: *Lactobacillus brevis*, *L. fermentum*, *L. plantarum* in food, hen and human faecal samples respectively and evaluated for *in vitro* probiotic attributes like resistance to low pH and high bile concentrations in simulated human intestinal conditions. The acid tolerance test was studied at pH 2.0 and 3.0 with 7.0 as control. The viable count for the acid tolerance test was obtained at an interval of 0, 1, 2 and 3 hrs respectively by standard plate count using deMan, Rogosa and Sharpe (MRS) agar after incubation at 37°C for 24 hrs. Isolates were subjected for bile tolerance test in MRS broth containing bile concentrations of 0% as control and 0.3, 0.5, 1.0 and 2.0% concentration as test followed by enumeration after 24 hrs of incubation on MRS agar. The maximum survival under simulated gastric juices at pH 2.0 for 3 hrs were shown by *L. plantarum* and *L. fermentum* while on the other hand tolerance to bile salts (0.3, 0.5, 1.0 & 2.0%) were shown by *L. fermentum, L. plantarum and L.brevis* respectively as shown in the following figure .

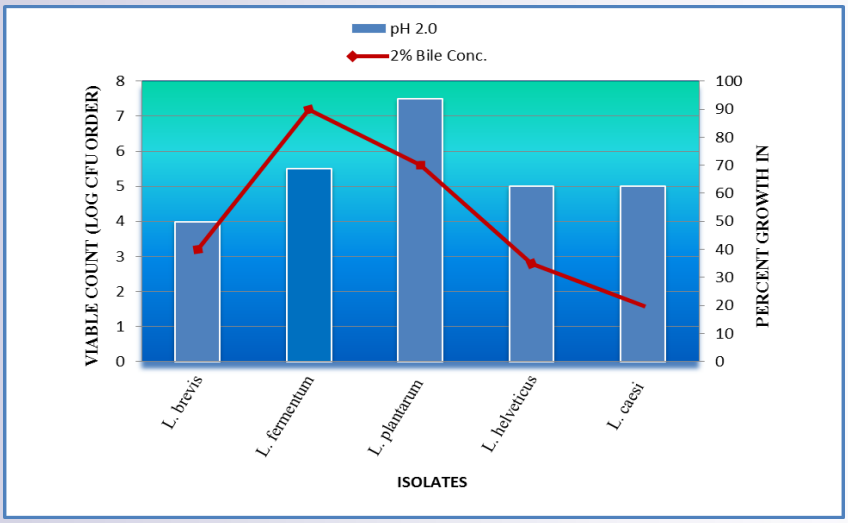

Figure showing comparative survival of Lactobacilli under simulated gastric juices at pH 2.0 and 2% bile concentration for 3 hours.

Remarkably, *L. plantarum* and *L. fermentum* of human faecal origin were able to survive at 2.0 pH upto 3 hrs. The data of this research suggest that the isolated acid and bile tolerant lactobacilli possess growth advantage under stress conditions and could be subjected further for selection of probiotic organisms include functional and technological safety aspects (i.e. antimicrobial activity, adherence, immune stimulation, resistance to phage etc.) before characterization with animal models.

A combination of probiotics and whey proteins enhances anti-obesity effects of calcium and dairy products during nutritional energy restriction in aP2-*agouti* transgenic mice

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Obesity is a major public health issue as it is related to several chronic disorders, including type-2 diabetes, high blood pressure, dyslipemia, cardiovascular diseases and cancer, among others. Previously authors demonstrated that dairy sources of calcium exert substantially greater anti-obesity effects than calcium carbonate. Compared with calcium carbonate, the use of dairy products as a calcium source induced a significantly greater attenuation of diet-induced obesity and acceleration of weight and fat loss during energy restriction in both mice and humans. Thus, dairy products appear to contain additional bioactive compounds that provide an attenuation of adiposity beyond that found with calcium. Therefore, present study was devised in aP2-agouti transgenic mice to assess anti-obesity effects of probiotic fermented milk.

Lactobacillus rhamnosus GG, Lactobacillus paracasei TMC0409, *Streptococcus thermophilus* TMC1543 and whey proteins were used to prepare fermented milk. For the experiment, aP2–*agouti* transgenic mice were pretreated with a high-sucrose/high-fat diet for 6 weeks to induce obesity. The obese mice were fed a diet containing 1·2 % Ca and either non-fat dried milk (NFDM) or probiotic-fermented milk (PFM) with nutritional energy restriction for 6 weeks. The animals were examined after the treatment for changes in body weight, fat pad weight, fatty acid synthase (FAS) activity, lypolysis, the expression levels of genes related to lipid metabolism, insulin sensitivity in adipocytes and skeletal muscle and the presence of biomarkers for oxidative and inflammatory stress in plasma. It was found that the PFM diet significantly reduced body weight, fat accumulation, and adipocyte FAS activity, and increased adipocyte lipolysis as compared with the effects of the NFDM diet ($P< 0·05$). The adipose tissue gene expression of 11β-hydroxysteroid dehydrogenase 1 (*11β-HSD1*) was significantly suppressed in mice that were fed PFM as compared with those that were fed NFDM ($P< 0·05$). PFM caused a greater up-regulation of skeletal muscle *PPARα*, *PPARδ*, uncoupling protein 3 (*UCP3*) and *GLUT4* expression and a significant decrease in the plasma concentration of insulin, malondialdehyde, TNF-α, monocyte chemotactic protein-1 and C-reactive protein as compared with the effects of NFDM ($P< 0·05$). Fermentation of milk with selected probiotics and supplementation of milk with whey proteins may, thus, enhance anti-obesity effects of Ca and dairy products by the suppression of adipose tissue lipogenesis, activation of fat oxidation in skeletal muscle and reduction of oxidative and inflammatory stress.

Source : British Journal of Nutrition / Volume 113 / Issue 11 / June 2015, pp 1689-1696

List of New Members of PAi

S.No	Name	E mail ID	Membership ID
Life Members			
1	Priyar Selvam Sellamuthu	periyar.india@gmail.com	371
Corporate Members			
1	Yakult Danone India Pvt. Ltd	neerja.hajela@yakult.co.in	
2	Shree Additives Pharma & Foods Pvt. Ltd.	vijay@shreeadditives.com	
3	Sarvotham Care Ltd.	mohan_krishna@sarvothamcare.com	
4	Microbax (India) Ltd.	nfo@microbax.com	
5	Mother Dairy	Anushri.Rastogi@motherdairy.com	
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1	C.P. Charles	ncrownson@abtfoods.com	372
Student members			
1	Bambam Kumar Singh	bambam.sgidt@gmail.com	376

ANNOUNCEMENT—UPCOMING CONFERENCE

As per an initiative taken by NISER, Bhubneswar, it has been proposed to hold the 3rd biennial PAi Conference with an International Symposium :

'STRESS, GUT MICROBIOME AND PROBIOTICS'

on 11th& 12th March, 2016 at Bhubneswar in joint partnership with National Institute of Science, Education & Research (NISER). This proposal was specifically considered to widen the coverage of probiotic science in frontier areas of research in other parts of the country as well. This is just the first announcement. Kindly wait for the next notification to get an update of the Conference with detailed technical program & other related activities by keep visiting our website.

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