# Probiotic Association of India

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#### From Editor's desk

Dear Esteemed Members,

#### Warm greetings!

The editorial board of Probiotic Association of India (PAi) welcomes you to the March edition of its newsletter and expresses sincere appreciation and thanks to all the authors who generously contributed in this newsletter. Probiotic science is a rapidly growing field and new probiotic foods are occupying the shelves of different stores everyday. As all of you know that India's highest statutory body "Food Safety and Standards Authority of India" have also operationalized standards on "Health supplements, Nutraceuticals, Foods for special dietary use, Foods for Medical purpose, Functional Foods and Novel foods", probiotics and prebiotics will now be regulated under these standards in India. Most of the spurious probiotic products containing unidentified bacterial strains will be out of shelves. The manufacturers of probiotics will now be required to adhere to the regulations set by FSSAI which will ensure consumers' confidence in the probiotic products. Since, most of the consumers and health professionals have little knowledge of the various probiotic strains used in the products, we need to create awareness and PAi has been dedicatedly working towards achieving this goal.

PAi is pleased to inform its readers that a workshop on "Probiotics in Clinical Practice" is going to be organized by PAi from 20<sup>th</sup> to 21<sup>st</sup> April, 2017. PAi is also going to organize 4<sup>th</sup> Biennial conference of PAi and International Symposium on 'Probiotic Therapy: Translating to Health and Clinical Practice' on February 16-17, 2018 at JLN Auditorium, All India Institute of Medical Sciences, New Delhi in association with AIIMS. The organizing secretary of the International Symposium is Dr. Rama Chaudhry. PAi would be very pleased to welcome participation of each and every member in the conference and showcase their work. Lastly, I acknowledge my sincere thanks to my colleague Dr. Rajeev Kapila who is working dedicatedly as a member of the editorial board and Ms Taruna Gupta for all the support rendered by them to shape this issue. It is because of their efforts that this issue is seeing the light of the day. Further, I request our readers to contribute in newsletters by sending us their valuable work in probiotic science.

I wish all our readers a great success in their endeavours in probiotic and gut microbiota science!

(Sunita Grover)
Chief Editor

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# Feeding probiotic *Lactobacillus rhamnosus* (MTCC 5897) fermented milk to suckling mothers alleviates ovalbumin-induced allergic sensitisation in mice offspring Vamshi Saliganti, Rajeev Kapila\*, Rohit Sharma and Suman Kapila Animal Biochemistry Division, ICAR-National Dairy Research Institute, Karnal, Haryana, India Corresponding author: Rajeev Kapila; rkapila69@rediffmail.com



The prevalence of allergic diseases in newborns continues to increase worldwide. It has been estimated that about 1–2% of the population and up to 8 % of children suffer from some type of IgEmediated food allergy. Several types of foods which include foods of plant origin such as peanuts, tree nuts, wheat and soya together with allergens of animal origin including cows' milk, egg, fish and shellfish are responsible for causing the majority of food allergies. The spectrum of food allergy symptoms may include flushing, urticaria, angio-oedema, laryngo-oedema, diarrhoea, nausea, vomiting, bronchospasm or hypotension. As there are no medical treatments currently available for curing food allergies, the best way to prevent unintended exposure to a food allergen is the complete avoidance of the offending foods. For various reasons, such avoidance may not always be possible, and in certain instances impossible. The neonatal period is often polarised to T helper (Th2) response at the time of birth, predisposing offspring to allergic disorders. Early infancy is a critical period for the development of immunological memory, where the T helper (Th) balance converts from the Th2-skewed immunity to Th1 cell-type response under the influence of genetic and environmental factors. Studies suggest that postnatal maturation of the immune system in atopic individuals is attenuated, and atopic infants show an imbalance in Th1/Th2 immune response by excessive IgE responses.

Passive immunity through the mother's milk is critical for immune system development of newborns. Probiotics have been proposed to harmonise Th1/Th2 imbalance in allergic conditions in adults. In the present study, the anti-allergic effects of feeding probiotic *Lactobacillus rhamnosus* fermented milk (PFM) either to dams during the suckling period or to their offspring after weaning individually or else in successive periods against ovalbumin (OVA)-induced allergy in newborns was analysed. After allergen sensitisation, physical symptoms of allergy, gut immune response, humoral immune response and cell-mediated response through interleukins were detected. Consumption of PFM by mothers and offspring showed a reduction (P <0·01) in physical allergic symptoms in newborns with an increase (P <0·01) in the numbers of goblet and IgA  $^+$  cells in the small intestine. Similarly, considerable (P <0·001) decreases in OVA-specific antibodies (IgE, IgG, IgG1) and ratios of IgE/IgG2a and IgG1/IgG2a in the sera of newborn mice were recorded.

A decrease in IL-4 and an increase in interferon-γ levels further confirmed the shift from Th2 to Th1 pathway in PFM-fed mice. It is logical to conclude that the timing of PFM intervention in alleviating allergic symptoms is critical, which was found to be most effective when mothers were fed during the suckling period.

Source: British Journal of Nutrition (2015), 114:1168-1179



# Induction of regulatory T-Cells: A role for probiotics and prebiotics to suppress autoimmunity

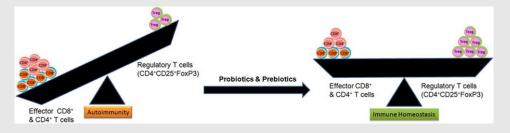
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Regulatory T cells (T-regs) are comprised of a heterogeneous population of cells that play a vital role in suppressing inflammation and maintaining immune tolerance. Given the crucial role of T-regs in maintaining immune homeostasis, it is probably not surprising that many microbial species and their metabolites have the potential to induce T-regs. There is now great interest in the therapeutic potential of probiotics and prebiotics based strategies for a range of autoimmune disorders. The review published by Dwivedi *et al.*,2016 summarises recent findings concerning the role of probiotics and prebiotics in induction of T-regs to ameliorate the autoimmune conditions. In addition, the article explains the different mechanisms of T-reg induction and function by these probiotics and prebiotics, based on the available studies till date. The article further proposes that induction of T-regs by probiotics and prebiotics could lead to the development of new therapeutic approach towards curbing the autoimmune response and as an alternative to mental immunosuppressive drugs.



Source: Autoimmunity Review (2016),15(4): 379-392.

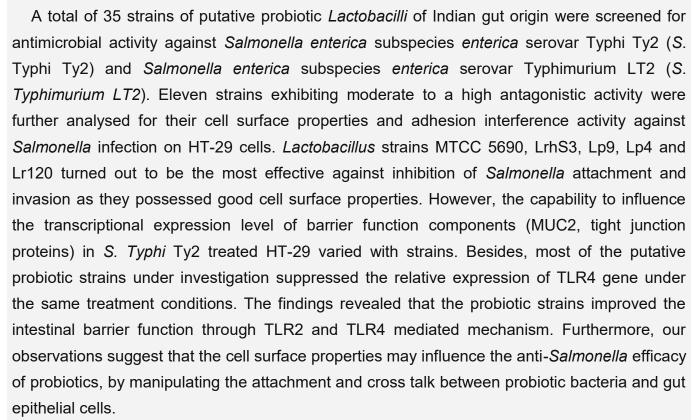
Interaction between putative probiotic *Lactobacillus* strains of Indian gut origin and *Salmonella*: Impact on intestinal barrier function

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Source: LWT- Food Science and Technology (2016),doi.org/10.1016/j.lwt.08.021.





# Diversity of putative lactic acid bacteria and their efficacy as probiotics on freshwater fish

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Although the applications of lactic acid bacteria (LAB) as probiotics have been well established for human and terrestrial animals, but its application in freshwater aquaculture is relatively recent. Recently indiscriminate use of antibiotics to control fish diseases has created awareness towards microbial interventions like indigenous or putative LAB in the form of probiotic treatments for sustainable and environment friendly aquaculture. There is also scanty information about the distribution of such bacteria in freshwater fish species. The present study therefore, focused on the diversity of putative LAB and their efficacy as probiotics on freshwater fish. Seventy six strains of LAB were isolated from intestines and identified by phenotypic tests and 16S rDNA gene sequencing. Phenotypic characterization of the isolates allowed the identification of 18 clusters at 78% similarity level by Hierarchical cluster analysis. Functional evenness index (E value) a measure of phenotypic diversity, was found to be quite high (0.7 approximately) in most of the samples. 16S rDNA gene sequencing identified the isolates as different strains of Lactobacillus plantarum, Lactobacillus pentosus, Lactobacillus fermentum, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus brevis, Lactobacillus reuteri, Lactobacillus salivarius, Pediococcus pentosaceus, Pediococcus acidilactici, Weissella paramesenteroides, Weissella cibaria, Enterococcus faecium and Enterococcus durans. L. plantarum was found to be the dominating strain and LAB occurred more frequently in hindgut.

Most of the strains showed good survival in acid and bile tolerance tests and antimicrobial activity against fish pathogen *Aeromonas hydrophila*. Different bacteriocin producing genes were detected in several strains by PCR. Based on *in vitro* characteristics, a consortium of selected putative LAB was used as a dietary supplement and subjected to feeding trials to investigate the probiotic effect on *Labeo rohita* (rohu) with emphasis on growth performance, immune response and disease resistance against *A. hydrophila*.

At the end of the 30 days of experiment, probiotic fed group showed a significant improvement in growth performance along with increased respiratory burst activity of blood phagocytes and serum antiprotease activity level. Quantitative Real-time PCR showed significant up regulation of IL-10 gene in kidney, intestine and liver of probiotic treated group whereas TNF-α gene was significantly up regulated only in liver and intestine. *HSP70* gene was significantly up regulated in intestine but down regulated in liver on day 15. Challenge with *A. hydrophila* on day 30 of probiotic feeding showed a significant increase in survival percentage of treated (93.33%) over the control group (33.33%). Subsequent challenge after 20 and 40 days of withdrawal of probiotic showed higher survival percentage (60% and 40% respectively) in withdrawn group compared to control although difference was statistically insignificant. The consortium of putative probionts may serve simultaneously as an immunomodulation feed additive, useful for disease protection and growth enhancer in eco friendly freshwater aquaculture practices. However, feeding at regular interval with probiotic supplemented diet is suggested for a prolonged immunity.



Fig. 1 Agarose gel electrophoresis of PCR amplification products of bacteriocin Plantaricin genes (*plnA*, *plnB*, *plnC*, *plnD*, *plnEF*, *plnI*, *plnJ*, *plnK*, *plnG*, *plnN* genes, *plnNC8*) in *L. plantarum* SM33

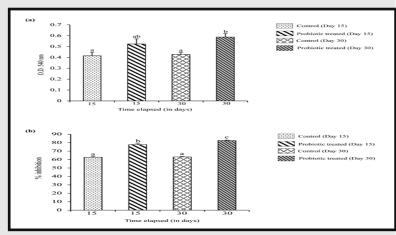


Fig. 2 (a) NBT activity of blood

(b) Antiprotease activity of serum from *Labeo rohita* fed with control diet and probiotic supplemented diet on day 15 and day 30 of the experiment. Data are expressed as mean  $\pm$  SE. Columns with different letters are significantly different (P < 0.05).

Source: Turkish Journal of Fisheries and Aquatic Sciences (2016), 16: 805-818
Aquaculture International (2017), DOI 10.1007/s10499-017-0122-5



# Mathematical modelling of dairy isolated probiotic organism Lactobacillus acidophilus (NCDC-13) in composite substrate Sangita Ganguly\* and Latha Sabikhi

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Probiotic organisms are well known for their multiple health beneficial effects like immunomodulation, anti-mutagenic, anti-carcinogenic, anti-diarrheal properties, etc. Most probiotic organisms are dairy isolates and their growth has been observed in dairy substrate. Very few attempts are made to observe the growth of probiotic organisms in other fermentation substrates such as cereals and combination of milk and cereals. Mathematical models are common tools for prediction of the growth, death and survival of microorganisms in certain substrates. The process of modelling usually begins with the first order models which are associated with the changes in the number of microorganisms with time and the information about the generation time, specific growth rate etc. can be obtained. The logistic equation is a most common and illustrative model of organism's growth dynamics and modified logistic models are widely used to describe the growth curves of organisms. Lactobacilli are very fastidious micro-organisms that require complex fermentable substrate. Substrate composition and environmental factors can affect the growth of organism, affecting the model parameters.

A multipurpose composite substrate was prepared by adding germinated pearl millet flour (4.73%) and Liquid Barley Malt Extract (3.27%) in Whey Skim Milk mixture (60:40). The mixture was heated at 95°C for 10 min and cooled to 37°C. The growth characteristics of dairy isolated probiotic organism *L. acidophilus* NCDC-13 (1% inoculum) were studied in the substrate at 37°C at 2 h intervals till death phase occurred. A mathematic model based on the following equation was developed to represent the cell growth and evaluated for its suitability.

$$X = \frac{X_0 X_{max} e^{(\mu_{max}t)}}{X_{max} - X_0 + X_0 e^{(\mu_{max}t)}} \dots (1)$$

where, X - cell concentration at time t,  $X_0$  – initial cell count,  $X_{max}$  - maximum attainable cell count,  $\mu_{max}$  - maximum specific growth rate predicted by the model and t – time. This equation represents both the exponential (X<X  $_{max}$ ) and the stationary phase (X=X  $_{max}$ ) of growth. The kinetic parameters of eqn (1) were estimated using advance nonlinear curve fitting function of software Origin 8.

Probiotic organisms grew very well in composite substrate due to presence of several nutrients. Fig. 1 represents the growth characteristics of NCDC-13 in composite substrate. An exponential growth phase of 12-13 h was observed for the organism in the optimised substrate and the stationary phase attained after 14 h of incubation. Since all fermentations were performed under no pH control, the organic acids formed via the metabolic pathways decreased the pH of the media. The pH and acidity at the end of exponential phase was 4.5-4.6 and 0.75-0.80% LA. Table 1 lists the kinetic parameters of the logistic equation estimated from the software Origin 8. The suitability in predicting the growth curve by logistic equations was validated by coefficient of determination (Adj. R² - 0.95) and reduced chisquare ( $\chi$ 2 - 0.311), respectively. Both these parameters (R²>0.95 and  $\chi$ 2<0.5) indicated the suitability of model for prediction of data. Additionally, the residuals obtained were random in nature, which is desirable. Based on these observations, the suitability of the logistic equation for modelling the growth curve in composite substrate was established. The logistic equation was also found suitable to express the growth of probiotic *Lactobacillus* sp. in cereal substrate (Charampopoulus *et al.*, 2002) and bifidobacterial strain in malt hydrolysate (Rozada-Sanchez *et al.*, 2008).

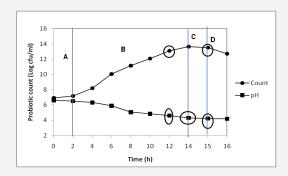


Fig. 1. Growth characteristics of NCDC-13 in composite substrate. Changes in probiotic count (log cfu/ml) and pH.

Table 1. Numerical values of estimated microbial growth parameters obtained from logistic model

Parameters	Value	Adj. R <sup>2</sup>	Reduced χ2
$X_0$	6.28 ± 0.44		
X max	14.65 ± 1.00	0.95	0.31
$\mu_{max}$	0.18 ± 0.04		

Where,  $X_0$ - Initial cell concentration (Log cfu ml<sup>-1</sup>),  $X_{max}$ - Maximum attainable cell concentration (Log cfu ml<sup>-1</sup>) and  $\mu_{max}$ -maximum specific growth rate (h<sup>-1</sup>).

#### Source:

- Charalampopoulos, D., Pandiella, S.S., & Webb, C. Growth studies of potentially probiotic lactic acid bacteria in cereal-based substrates. *Journal of Applied Microbiology* (2002),92: 851–859.
- Rozada-Sanchez, R., Sattur, A.P., Thomas, K., & Pandiella, S.S. Evaluation of Bifidobacterium spp. for the production of a potentially probiotic malt-based beverage. Process Biochemistry (2008),43(8):848–854.



Modulation of intestinal barrier function to ameliorate *Salmonella* infection in mice by oral administration of fermented milks produced with *Lactobacillus* plantarum MTCC 5690– a probiotic strain of Indian gut origin

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Probiotic Lactobacillus plantarum MTCC 5690, a probiotic strain of Indian gut origin and milk formulations produced with the same were explored as biotherapeutics by evaluating their functional efficacy against Salmonella infection in mice. The efficacy of milk formulations (fermented/unfermented) of MTCC 5690 for enhancement of intestinal barrier function was determined by monitoring the permeability and histopathology of the intestine. Salmonella infected mice fed with probiotic Dahi, fermented probiotic drink and sweetened fermented probiotic drink maintained the health and integrity of the intestinal epithelium as compared to those fed with PBS, milk, unfermented probiotic milk and Dahi. Our relative expression data revealed that the changes caused by MTCC 5690 in intestinal barrier function components were established through modulation of the key regulatory receptors (Toll-like receptor 2 and Toll-like receptor 4). The results suggest that fermented milks of MTCC 5690 could enhance the intestinal barrier defenses in enteric infection condition and, therefore, can be explored as a dietary-based strategy to reduce Salmonella infection in the human gut.

Source: Journal of Medical Microbiology (2016),65(12):1482-1493.

# Probiotic Microbes Sustain Youthful Serum Testosterone Levels and Testicular Size in Aging Mice

Theofilos Poutahidis, Alex Springer, Tatiana Levkovich, Peimin Qi, Bernard J. Van, Jessica R. Lakritz, Yassin M. Ibrahim, Antonis Chatzigiagkos, Eric J. Alm<sup>a</sup>, Susan E. Erdman <sup>b</sup>

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The decline of circulating testosterone levels in aging men is associated with adverse health effects. During studies of probiotic bacteria and obesity, the authors discovered that male mice routinely consuming purified lactic acid bacteria originally isolated from human milk had larger testicles and increased serum testosterone levels compared to their age-matched controls. Further investigation of histomorphometry using microscopy-assisted testicular tissue showed that mice consuming Lactobacillus reuteri in their drinking water had significantly increased seminiferous tubule cross-sectional profiles and increased spermatogenesis and Leydig cell numbers per testis when compared with matched diet counterparts This showed that criteria of gonadal aging were reduced after routinely consuming a purified microbe such as L. reuteri. The authors tested whether these features typical of sustained reproductive fitness may be due to anti-inflammatory properties of L. reuteri, and found that testicular mass and other indicators typical of old age were similarly restored to youthful levels using systemic administration of antibodies blocking pro-inflammatory cytokine interleukin-17A. This indicated that uncontrolled host inflammatory responses contributed to the testicular atrophy phenotype in aged mice. Reduced circulating testosterone levels have been implicated in many adverse effects; dietary *L. reuteri* or other probiotic supplementation may provide a viable natural approach to prevent male hypogonadism and side-effects of traditional therapies, and yield practical options for management of disorders typically associated with normal aging. These novel findings suggest a potential high impact for microbe therapy in public health by imparting hormonal and gonad features of reproductive fitness typical of much younger healthy individuals.

Source: PLOS One (2014), doi.org/10.1371/journal.pone.0084877

#### Presentation of work at international Conference



Namita Rokana, Ph.D., Dairy Microbiology Division, ICAR-NDRI, Karnal Participated at International Conference of International Association for Probiotics and Prebiotics-Student Fellow Association (ISAPP-SFA) -2015 annual meeting held at Washington DC on 19-21 May 2015 and presented the paper for knowledge exchange session entitled "Impact of probiotic fermented milk formulations on amelioration of Salmonella infection through modulation of intestinal barrier function."

A total of 35 indigenous strains of probiotic *Lactobacilli* were subjected to a preliminary screening based on their antimicrobial activity against Salmonella Typhi Ty2 and Salmonella Typhimurium LT2. Eleven strains exhibiting moderate to high antagonistic activity were shortlisted for investigating their cell surface properties. L. plantarum Lp91, Lp77, Lp9, Lp4, L. fermentum Lf2, L. rhamnosus LbS3, and L. reuteri Lb120 demonstrated strong ability to coaggregate with LT2. The degree of exclusion of pathogen from HT-29 cell monolayer also varied with strains. Based upon these properties, Lp91 was finally selected to investigate its effect on intestinal barrier function (IBF) against Salmonella infection in a mice model. Considering the fact that food matrix could affect the functionality of probiotics, fermented and unfermented milk formulations of Lp91 were examined for their desired effect on IBF. Measurement of intestinal permeability revealed that fermented food groups effectively maintained the health and integrity of intestinal epithelium as compared to those fed with unfermented food. The mechanism of protection against infection was demonstrated by the modulation of key regulatory receptors TLR2, TLR4 and by up-regulating the expression of the tight junction genes along with secretory components; mucin-2, β-defensin 2 and cathalicidin. The results indicate that consumption of *L. plantarum* Lp91 in the form of fermented dairy food could increase the functioning of intestinal barrier in normal health as well as enteric infection conditions. Hence, Lp91 based fermented milk formulations have the prospects to be explored as the preventive and therapeutic intervention to protect human gut against Salmonella infection.

#### **Awards and Achievements**

# Indian National Science Academy (INSA) Indo-Australia Researcher Fellowship Programme 2016-17

Indian National Science Academy, New Delhi has awarded Indo-Australia fellowship to Dr. Santosh Kumar Tiwari, Department of Genetics, Maharshi Dayanand University, Rohtak. This prestigious fellowship has been awarded to 20 candidates out of 174 applications received from all over India. Under this programme, Dr. Tiwari will visit world's renowned crystallographer Prof. Fasseli Coulibaly, Monash University, Australia to conduct research on structure-function relationship of bacteriocin of Lactobacillus. He will also visit Professor Sanjay Patole laboratory, KEM hospital, Perth to understand the therapeutic applications of these bacteriocins and deliver a lecture on "Therapeutic applications of bacteriocins of probiotic lactic acid bacteria". Bacteriocins have shown great advantages over clinical antibiotics for least resistance developments towards pathogens.



#### **Best Thesis Award Nomination**

**Dr. Prasad Subhash Patil student** of Ph.D. Dairy Microbiology, ICAR-NDRI was nominated to present his work entitled "**Production and Characterization of Alpha-glucosidase and Dipeptidyl Peptidase-IV Inhibitory Milk Bioactive Peptides Using Lactobacillus spp**" for best doctoral thesis award during academic week of 15<sup>th</sup> convocation of ICAR-NDRI held on 4<sup>th</sup> March, 2017.

#### **Best Thesis Award Winner**

**Surya Kant student** of M.Sc. Animal Biochemistry, ICAR-NDRI was nominated to present his M.Sc. work entitled "**Evaluation of Pediocin Containing Fermented Cheese Whey for Reducing total Microbial Load in Raw Buffalo Milk**". He was conferred "**Best Thesis Award**" in the processing for the year 2015-2016 during academic week of 15<sup>th</sup> convocation of ICAR-NDRI held on 4<sup>th</sup> March, 2017.







#### "Announcement & Upcoming Probiotics Events"

1. 4<sup>th</sup> Biennial conference of PAi and International Symposium on 'Probiotic Therapy: Translating to health and clinical practice' will be held on February 16-17, 2018 at JLN Auditorium, All India Institute of Medical Sciences, New Delhi in association with AlIMS. Organizing Secretary: Dr. Rama Chaudhry.

For more details, kindly go through: http://www.probioticindia.com

2. Conference series LLC welcomes and invites all the participants from over the world to attend The 6<sup>th</sup> International Conference and Exhibition on Probiotics, Functional and Baby Foods to be held on October 02-03, 2017 in London, UK.

For more details, kindly go through: http://probiotics.conferenceseries.com

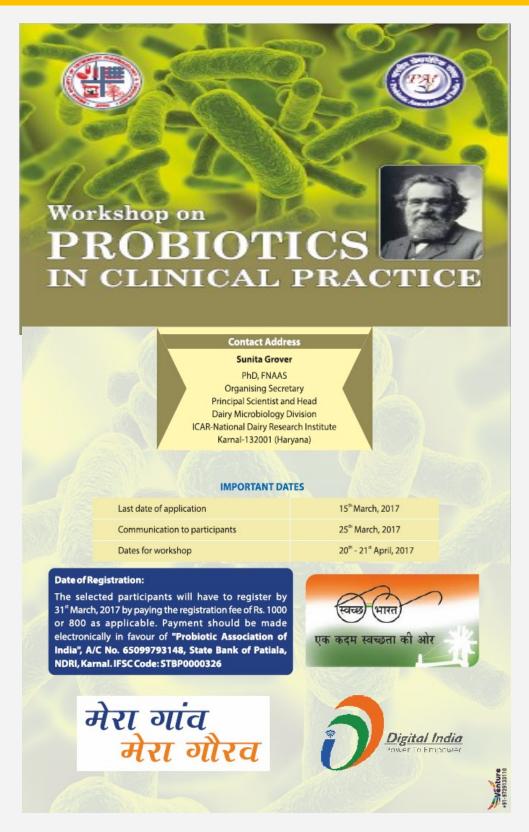
3. "14<sup>th</sup> International Conference on Clinical Nutrition" is going to be held on July 27-29, 2017 Rome, Italy. A leading forum for leaders in Academia, Pharmaceutical and Industry, contract research organizations, API manufacturers, domestic and international regulatory agencies and executive directors of leading pharmaceutical companies and experts. The conference topics include: Nutrition, Obesity and Diabetes, Public Health Nutrition, Nutritional Disorders and Treatment, Nutrition in Women and Postmenopausal diet, Pediatric Nutrition and Obesity, Foods and Nutritional Supplements, Diabetes Nutrition, Sports Nutrition, Current research in Nutrition, Nutritional Physiology and Food Biochemistry and Food sciences and Probiotic Nutrition and Enteral nutrition etc.

For more details, kindly go through: http://clinicalnutrition.conferenceseries.com

4. 13<sup>th</sup> International Congress on Advances in Natural Medicines, Nutraceuticals & Neurocognition which is going to be held in July 27-28, 2017 in Rome, Italy. Nutraceuticals 2017 conference is a leading forum for nutrition & dietary professionals, health care researchers, elite professors, students and business delegates from Nutraceutical and Natural Medicine manufacturing industries who can share their Cutting-edge technologies and research experience by giving lectures on various Sessions/Tracks.

For more details, kindly go through: http://nutraceuticals.conference.blogspot.in/

#### " Announcement & Upcoming Probiotics Events



#### "List of New Members of PAi (October, 2016 - March, 2017)"

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4	Jugsharan Singh Virdi	virdi_dusc@rediffmail.com	428	
5	Narendra Kumar	narendra289186@gmail.com	429	
6	Monica Gulati	monikagulati14@gmail.com	430	



Life Membership Fee : Rs. 3500 (Ten Years)

Student Member : Rs. 500 annually

Ordinary Member : Rs. 1000 annually

Institutional Membership : Rs. 10,000 annually

Corporate Membership : Rs. 25,000 annually

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