



# Probiotic Association of India

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## From Editor's Desk

Dear All,

Special Greetings from the editorial board of Probiotic Newsletter!!!

Hope, all the esteemed members of PAi and viewers of our website must be going great guns with excellence and new accomplishments both in their professional careers and domestic front. As you all know, world is moving rapidly with new developments and so does the Probiotics research which has now virtually entered into the era of 'biomics' and translation research in a big way across the world including our country. Many of the human microbiome projects are now running in full swing and their outcome is expected soon to unravel many of unresolved intricacies in understanding the role of gut flora and their novel functions. These new initiatives in Probiotics research and development at the global and international level are likely to generate very useful information on gut microbiomes of different countries/regions at genomics, transcriptomics, proteomics, metabolomics and metabonomics level for the benefit of human population from health and nutrition perspectives. In the backdrop of this wealth of

knowledge accumulated out of these initiatives, the 5th issue of PAi Newsletter has been specifically dedicated to metagenome of gut microbiota, and the massive genome sequencing data of new probiotic strains have already been launched on the NCBI database as public domain. With the growing awareness and knowledge on different domains of Probiotic world, the interest of our readers has also started increasing day by day. This can be reflected from an unprecedented response received from PAi members who had contributed their inputs in very large number, this time. We duly acknowledge our appreciation for all the contributors of these interesting articles which were accepted after undergoing critical review from the editorial board. We hope, based on this overwhelming response, more and more people will be prompted to share their updated knowledge and contributions made in this subject from their respective laboratories and participate in improving the quality and standard of our Newsletter in our upcoming issues. Wish you all the very best and have a wonderful quality time ahead. PAi also wishes its readers Happy Festive Season and a very Happy Diwali!!!

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## Development of PCR Based Method for Quantification of Selected Microflora in Dahi

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The availability of culture-independent tools that enable a detailed investigation on the microbial biodiversity of fermented food system is the need of the hour for food microbiologists. In the present study, a real-time PCR system was standardized after designing 16S rRNA targeted genus-specific primers for quantification of selected lactic acid bacteria from dahi. We designed four primer pairs specific for four predominant genera found in dahi samples i.e. *Lactobacillus*, *Streptococcus*, *Lactococcus* and *Leuconostoc*. The main objective was to standardize a multiplex assay for the enumeration of all the four genera as well as to correlate the data from traditional enumeration technique. The genotypic real time PCR technique with Real Time PCR calibration curves of pure cultures gave correlation coefficient,  $R^2 > 0.995$ . A total of 15 spiked samples were prepared using the four genera in combination with one, two and three cultures ranging from a concentration of 0.25% to 1% in the samples. The standard curves generated for the absolute quantification were of good quality displaying  $R^2$  values  $> 0.99$ . Samples were simultaneously enumerated using plate counts on specific media for all the four genera. The traditional plate counts and real time PCR counts for the 15 spiked samples showed a highly significant correlation ( $p > 0.01$ ). Hence, we can propose that the real-time PCR assay developed in this study was comparable to traditional plate counts and can be applied to enumerate the dominant bacterial species found in fermented foods like dahi. On comparing the counts for a particular genus in various combinations, lactobacilli counts obtained using real time PCR correlated significantly with plate counts when used in combination with three cultures while *Leuconostoc* sp. correlated well in combination with one and two cultures. Streptococci and lactococci assays showed non-significant differences in simplex and multiplex assays reflecting the primers specificity and precise assay conditions. The quantification obtained by real time PCR closely correlated with plate count quantification and could be useful for monitoring complexity, diversity and dynamics of fermented product dahi.

**Metagenome and metabolism: the tissue microbiota hypothesis.**

Burcelin, R., Serino, M., Chabo, C., Garidou, L., Pomié, C., Courtney, M. and Bouloumié, A. 2013. Metagenome and metabolism: the tissue microbiota hypothesis. *Diabetes, Obesity and Metabolism*, 15(s3), 61-70.

## **Application of Resolvase based in vivo expression technology (RIVET) for studying induction of bacteriocin genes in complex murine gut environment**

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Establishing the mechanisms by which microbes interact with the host environment is a major challenge for scientists. In this regard, a pioneering technology is Resolvase based in vivo expression technology that can be used to capture nearly all bacterial promoters upregulated during a microbe-environment interaction. Absolute confirmation of *L. rhamnosus* GG (LGG) bacteriocin production capabilities is not yet established. The main objective of this study was to investigate whether the probiotic strain LGG's bacteriocin encoding genes are induced inside the murine GIT. Bioinformatic tools led to the findings that LGG contains one Class IIa bacteriocin i.e. lantibiotic belonging to the pediocin like family (LGG\_02400) and class IIb (LGG\_02391-02392). With this information, a 200 bp genomic fragment containing the putative promoter of the genes LGG 02385-LGG\_02386 which encoded for putative bacteriocin export ABC transporter was amplified and the fragment was cloned at the XhoI-BglII site upstream of the promoterless cre gene in plasmid pCMPG5350, resulting in plasmid pCMPG5367. This plasmid was subsequently transformed to the reporter strain CMPG5340-RifR, containing the loxP- flanked TcR marker gene by electroporation. For analysis of the induction of bacteriocin promoters, two mice received a 100µl oral dose ( $2 \times 10^{10}$ cfu) of these freshly prepared bacterial suspensions by intragastric administration on 2 consecutive days. It was observed that less than 0.5% of the colonies from the 48 h time point were tetracycline sensitive. This is probably related to the fact that LGG showed a low rate of cell division in the murine GIT and one cell division is needed for each Cre-mediated excision. From the above results, it can be concluded that the expression of the promoter is very low and only in few cells i.e. a stochastic expression. It can be said that the expression of the promoter is not in a constitutive form. These, results support that bacteriocin may serve as an adaptation factor for the survival and persistence of *Lactobacillus rhamnosus* GG inside the gastrointestinal tract of mice.

**Richness of human gut microbiome correlates with metabolic markers.**

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G. and Pedersen, O. 2013. Richness of human gut microbiome correlates with metabolic markers. *Nature*, 500 (7464), 541-546.

## **Genomic analysis of dairy starter culture bacterium *Streptococcus thermophilus* MTCC 5461 revealed probiotic potential**

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The lactic acid bacterium (LAB) *Streptococcus thermophilus* is widely used as a starter culture for the production of dairy products. Whole genome sequencing is expected to utilize the genetic basis behind metabolic functioning of LAB, for development of their use in biotechnological and probiotic applications. *Streptococcus thermophilus* MTCC 5461 was isolated from curd. We sequenced the whole genome of the strain by 454 GS-FLX titanium and Ion Torrent PGM. In total, 1,764 coding sequence (CDS) regions and 78 RNA genes were observed. Of 78 RNA genes, 59 coded for tRNA, 13 for rRNA, and 6 for 5S RNA. Fifty nine tRNA represented all the amino acids and were non redundant for histidine, cysteine, and aspartate. The CDS regions were slightly fewer than in *S. thermophilus* CNRZ 1066 (1,969). Metabolic reconstruction subsystems were assembled to create a metabolic reaction network for *S. thermophilus* MTCC 5461. We performed comparative genome analysis using local BLAST and RDP for 16S rDNA comparison and RAST server for functional comparison against the published genome sequence of *Streptococcus thermophilus* CNRZ 1066. The whole genome size of *S. thermophilus* MTCC 5461 is of 1.62 Mb size with a GC content of 39.3%. The strain can be concluded to be non-pathogenic and have a stable genome structure based on the genes present. From the genome study and annotation results obtained, it can be interpreted that the absence of surface exposed proteins and lack of many coding sequences related to carbohydrate metabolism confirmed that the strain was non-virulent. The presence of sequences for genes like RecQ, sortase, and absence of PsaA, PsaC like virulence related genes in the genome content reflected its metabolism, biosynthetic capabilities, and adaptability to environments. Streptococcal virulence-related genes are either inactivated or absent in the strain. Genome of *S. thermophilus* strain revealed the presence of probiotic properties like acid tolerance, beta galactosidase activity, bacteriocin production and adherence to intestinal cells thus appearing as promising candidate for further investigation through biochemical and clinical studies to elucidate potential health benefits and application as a novel functional probiotic starter in the food industry.

**Summary of the article “Genomic analysis of dairy starter culture *Streptococcus thermophilus* MTCC 5461” , J Microbiol\_Biotechnol. 2013 Apr;23(4):459-66 by Prajapati JB, Nathani NM, Patel AK, Senan S, Joshi CG.**

## **Microencapsulation of Probiotics and Utilisation in Dairy based Foods**

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MVSc (LPT)

Probiotics dairy foods are very popular among the consumers for their health aspects beyond the palatability. Due to their promising health beneficial effects, they have been incorporated into a wide range of products. But, the viability of probiotics in fermented dairy foods as well as in gastrointestinal conditions is in very minimal level, which has encouraged various researchers to develop innovative methods to improve the viability of probiotics. Microencapsulation of probiotics is one of the approach currently receiving considerable attention.

Micro encapsulation is an inclusion technique for entrapping bio-active compounds such as probiotic bacteria, folic acid and enzymes into a polymer matrix that may be coated by one or more semi-permeable polymers, by the virtue of which the encapsulated substance become more stable than the

Microencapsulation has become a recent tool for protecting and delivering bio-actives in the development of functional foods. It is often mentioned as a way to protect bacteria against severe environmental factors.

free one. Microencapsulation of probiotic bacteria can be used to enhance and improve the viability during processing and also in gastrointestinal tract. It is the versatile technology which offers great potential for the application in dairy industry. This technology promises to preserve the sensory attributes like flavour, body and texture in the product incorporated. It can be used to produce starter cultures with higher viability, controlled release of flavour enhancing enzymes to accelerate cheese ripening and improved stability of final product during processing. Microencapsulation technology can be used to maintain the viability of probiotic bacteria during food product processing and storage. New prospects can be explored, using encapsulation technology for preparing probiotic products with greater shelf life, superior and more acceptability in the modern consumer market.

**Functional metagenomics reveals novel pathways of prebiotic breakdown by human gut bacteria.**

**Cecchini, D. A., Laville, E., Laguerre, S., Robe, P., Leclerc, M., Doré, J. and Potocki-Véronèse, G. 2013. Functional Metagenomics Reveals Novel Pathways of Prebiotic Breakdown by Human Gut Bacteria. *PloS one*, 8(9), e72766.**



## **Probiotics as potential biotherapeutics for management of type 2 diabetes**

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The incidence of diabetes has been increasing steadily worldwide at a regular pace with prevalence of type 2 diabetes (T2D), affecting almost all the major sections of the society. Inflammation plays a critical role in pathogenesis of T2D and other metabolic disorders (Fig. 1). Any therapy targeting inflammation can prove to be an effective approach for management of diabetes. Some of the probiotic strains are known for their rich anti-inflammatory potential and hence can serve as effective biotherapeutic agents against inflammatory metabolic disorders. Possible mechanisms by which probiotics confer their health promoting effects have been illustrated in Fig. 2. The connection between gut flora, energy homeostasis, inflammation and its role in pathogenesis of metabolic disorders is being increasingly recognized. Several recent reports have demonstrated

a link between altered gut microbiota to the development of obesity, insulin resistance, and diabetes. Any therapy targeting modulation of this microbial dysbioses, possibly by dietary intervention can be an alternative to ongoing toxic and costly therapeutics. Few attempts have been made in this direction wherein anti-diabetic functionality of different probiotic strains have been explored and postulated their beneficial effects through, although not limited to conservation of primary

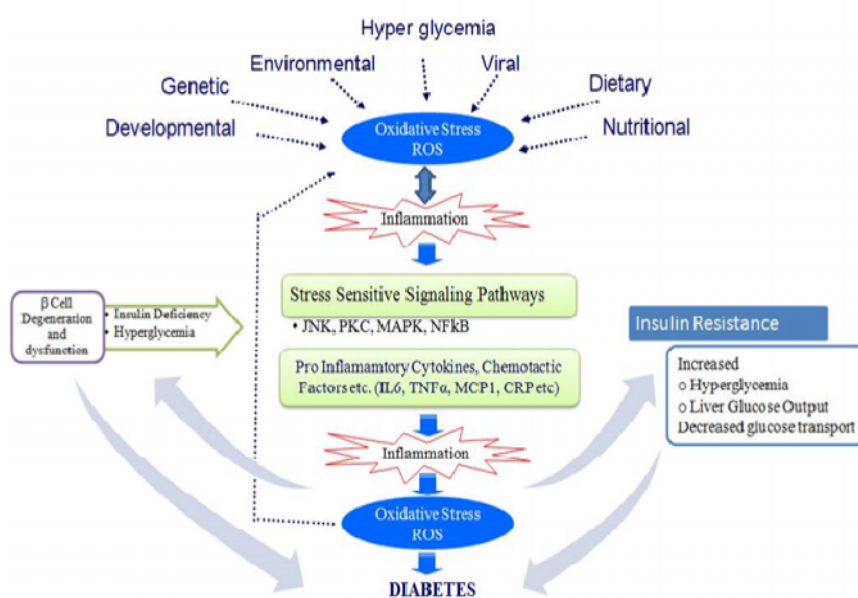


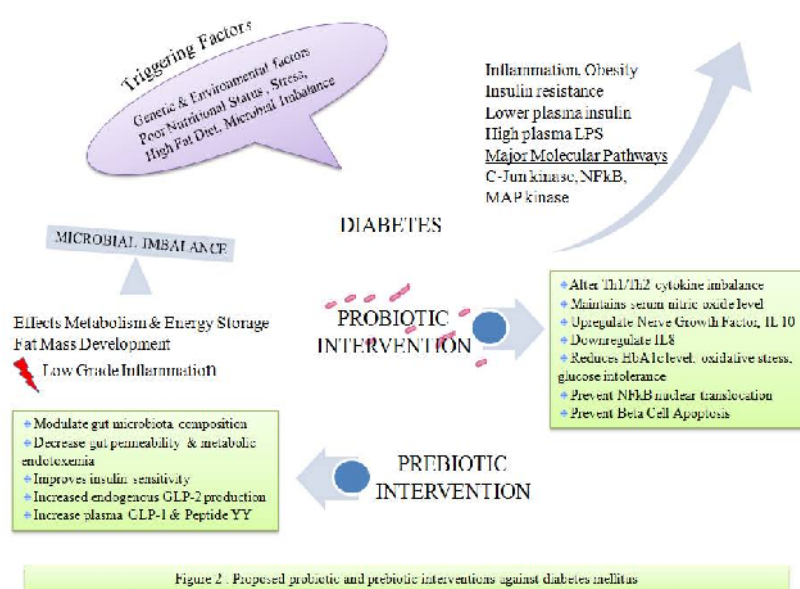
Figure 1 : Pathophysiology of Diabetes Mellitus

Continued to 7th

**For more detail refer : Panwar H, Rashmi HM, Batish VK, Grover S. 2013. Probiotics as potential biotherapeutics in the management of type 2 diabetes – prospects and perspectives. Diabetes Metab Res Rev., 29: 103-112.**

bile acids, decreased plasma glucose, inhibition of pro-inflammatory cytokines and transcription factors, inhibition of cell death, altering the imbalance of Th1/Th2 cytokines and differential stimulation of dendritic cells. From the initial leads that emerged from the limited studies on antidiabetic potentials of probiotic strains, it can be concluded that probiotic interventions directly or indirectly through food formulations do have the prospects to be explored as a novel

therapy in the treatment of T2D. However, more in depth mechanistic studies need to be conducted in appropriate animal models to understand the exact mode of action of the proven probiotic strains to manage this high priority chronic disease with serious health implications .



### Announcement

*Sixth International Conference on "Fermented Foods, Health Status and Social Well-being" will be organized by Swedish South Asian Network on Fermented Foods in collaboration with Anand Agricultural University and Lund University, Sweden during 6-7 December 2013 at AAU, Anand (Gujarat). The theme for the current conference is Novel Fermented Functional Foods for Prevention of Major Non Communicable Diseases. Apart from scholars, academicians and professionals working in food and biotech industries, this conference will be apt for nutritionists, physicians and pharma people too. Apart from technical sessions involving lead papers and young scientists presentations, the conference will have special industry forum session, panel discussions and poster sessions. Last date for sending abstracts is 30th October 2013. For details, please visit [www.fermented-foods.net](http://www.fermented-foods.net), [www.aau.in](http://www.aau.in) or send mail to [jbprajapati@gmail.com](mailto:jbprajapati@gmail.com)*

## Use “me” and “I” will guard your health

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Modern society is suffering from a number of diseases due to changing lifestyles and eating habits. Diabetes and obesity, both are silent assassins, that are tickling very fast like a time bomb affecting millions of people worldwide and their quality of life. The principal driver for the increase of type 2 diabetes mellitus (T2DM) is thought to be the worldwide rise in the prevalence of obesity. The increasing upper body obesity is accompanied by a progressive increase in the glucose and insulin resistance. Different hormones regulate lipolysis and contribute to release of free fatty acids (FFA) from abdominal adipocytes into the portal system. These have a detrimental action on the uptake of insulin which results in increased gluconeogenesis, production of glucose by the liver and systemic dyslipidaemia. Initially, the  $\beta$ -cells of the pancreas compensate for these processes by producing more insulin but at the end, failure of the cells and the development of hyperglycemia take place leading to T2DM. Development of inhibitors for nutrient digestion and absorption specially, inhibition of enzymes involved in gastrointestinal mechanisms are some of the promising strategies of treatment. This could be an alternative to other pharmacological approaches for the treatment of various diseases/ disorders. Inhibition of dietary carbohydrate and lipid absorption/ digestion via inhibition of pancreatic, salivary amylases/ lipases and intestinal  $\alpha$ -glucosidases is emerging as promising approaches for controlling diabetes. Microorganisms are regarded as man's best friends and worst enemies, hence may be used for the inhibition of enzymes involved in obesity and diabetes, and/ or as a source of inhibitors which could be used as therapeutic agents. A variety of enzyme inhibitors from plants, microbes, food-stuffs like fermented foods, vegetables, grains, spices, fruits have been isolated, screened and evaluated under in vitro and in vivo conditions. Till date, almost all the commercially available inhibitors are the secondary metabolic product of microorganisms or the end products of biotransformations. It is suggested that probiotics can be used (directly/indirectly) for the inhibition of these enzymes, which could be the new players.

### **Dietary intervention impact on gut microbial gene richness.**

**Cotillard, A., Kennedy, S. P., Kong, L. C., Prifti, E., Pons, N., Le Chatelier, E. and Ehrlich, S. D. 2013. Dietary intervention impact on gut microbial gene richness. *Nature*, 500(7464), 585-588.**



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