Six monthly Probiotic News letter

September, 2012– 3rd Issue

Probiotic Association of India

Probiotics for Human health –New Innovations and Emerging Trends

Inside

From Editor's desk

With profound greetings to our online viewers / readers, the editorial board of Probiotic Newsletter series is immensely pleased to launch the third issue of Probiotic Newsletter. As you may be aware that PAi had recently organized its Ist Annual conference along with an International symposium with the theme title "Probiotics for Human Health – New Innovation and Emerging Trend" on 27th and 28th Aug., 2012 at India Habitat Centre, Lodi Road, New Delhi. Since, this conference was the first under the auspices of PAi event and turned out to be an instant success from scientific perspective. This issue has been dedicated primarily to some of the salient scientific activities and glimpses that emerged from the conference program during its two days delebrations for benefit of those of PAi members who were not able to attend the conference due to their other commitments. We hope, our esteemed viewers would find this special issue more exciting, useful to keep their interest on probiotics and probiotic foods alive to garner maximal health benefits from these. Beside this, this issue also includes coverage of the company profile of Shree Additives Pharma and Foods, another corporate member, a latest addition to PAi fraternity to keep up our tradition of developing a good linkage with the industry. Meanwhile, the editorial board calls upon other corporate PAi members also to keep sending us their inputs for incorporating into the next issue of Probiotic Newsletter. Furthermore, we would also like to share that after the conduct of Ist Annual conference of PAi, the response from industry and students and faculty of central and state universities and research institutions for becoming corporate and lifetime members has been quite overwhelming and we now expect dramatic boost in PAi membership campaign.

We wish our readers very best of festive season ahead!

"Happy Diwali"

Conference highlights

1st Annual Conference of PAi and International Symposium on "Probiotics for Human Health- New Innovations and Emerging Trends" was organized by Probiotic Association of India was organized on 27th-28th August, 2012 at India Habitat Centre, Lodi Road, New Delhi. The conference was attended by about 200 participants from India and abroad working on different aspects of probiotics research and product development for human health applications. The conference was inaugurated by Mr K. Chandramouli, Chairperson, FSSAI, who in his inaugural address highlighted the initiatives being taken at FSSAI for regulating the quality and safety of processed foods including the value added functional foods and formulations supplemented with probiotics to confer their beneficial effects on human health and well being. He called upon the probiotic fraternity in the country to come forward and rise to the occasion to launch probiotic movement in the country for the benefit of the consumers and the society through their innovative R&D efforts and advise the food authority how to carry forward probiotic foods in the Indian market in the context of regulatory guidelines on probiotics recently launched by ICMR and DBT. Dr. Seppo Salminen, an authority on probiotics from Finland delivered the key note address. The main attraction of the conference was the presentations made by eminent speakers of International repute both from India and abroad in their respective areas of interest in probiotics in different plenary and technical sessions. It was followed by a session on panel discussion on some key issues that emerged from the technical presentations which were deliberated at length through extensive brainstorming. The outcome of these brainstorming sessions formed the basis for formulating some crisp recommendations for taking follow up action at the government level which eventually could help in formulating a national policy on probiotics in the country.

1st Annual Conference of PAi and International Symposium on "Probiotics for Human Health - New Innovations and Emerging Trends"

Awardees

Oral Young Researcher Awardees

Mr. Himanshu Kumar

Dr. Raj Kumar Duary

Ms. Suja Senan

Poster Awardees

Mr. Bhupesh Kumar Thakur

Mr. Akhilesh S. Dhanani

Ms. Aparna SV &

Ms. Divyashri G

Exploring microbial diversity of Kutajarista (an ayurvedic fermented biomedicine) for targeted isolation and characterisation of *Lactobacillus* spp. for probiotic attributes

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Introduction:

Ayurveda is a traditional system of medicine followed in India for centuries. Due to increasing side effects and complications by the repetitive use of antibiotics, now there is increased interest and application of natural or herbal remedies. Kutajarista is one of the most widely studied ayurvedic preparation, popularly known for its role in the treatment of gastrointestinal diseases like amoebic dysentery, piles and intestinal parasite infestation. In this study, we have explored microbial diversity of Kutajarista at different fermentative stages by 16S rRNA gene clone library approach. *Lactobacillus* spp. isolated from Kutajarista were subjected to *in vitro* and *n vivo* tests for probiotic attributes. Stability of isolated *Lactobacillus* spp. was also explored in Kutajarista formulation, for designing non- dairy probiotic fermented beverage.

Methods and Results:

Physicochemical analysis of Kutajarista samples collected at different fermentation time points showed that pH varied from 3.62 to 4.24 and acidity decreased from 0.302to 0.4301.Microbial diversity increased after 8th day of fermentation but gradually decreased with only 3 operational taxonomic units (OTU's) recovered at the saturation of fermentation. Like most of the fermentation processes,*Lactobacillus* spp. was found at initial time point of fermentation. To assess probiotic properties, *Lactobacillus plantarum* was found to be tolerant to pH 2, 0.3% bile concentration and simulated gastric juice. *L. plantarum* was also found to be adherent to intestinal epithelial HT-29 cell line. *In vivo* feeding trial in BALB/c mice also provided evidence that *L.plantarum* colonizes mouse gut as revealed by FACS and confocal analysis. In mouse gut, the maximum colonization was found to occur at colonic part of the intestine.

In concordance to earlier reports, Cell free supernatant (CFS) of *L. plantarum* was also antagonistic to enteric pathogens like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Aeromonas veronii* and clinical isolates of *P.aeruginosa* and *E. coli*. The preventive role of cell free supernatant of *L. plantarum* in amelioration of cytotoxicity caused by *Aeromonas veronii* was established in Vero cell line. In addition, *A. veronii* CFS caused disruption of tight junction proteins ZO-1 and actin in MDCK cell line, which was prevented in cells pre-incubated with CFS of *L. plantarum* as revealed by immunostaining. Real time PCR was done to evaluate the immunomodulatory role of *L. plantarum* in mouse macrophage RAW cell line. CFS of *L. plantarum* was also found to be anti inflammatory as it reduced the expression of pro-inflammatory markers like TNF-α, IL-1β etc., activated by *A. veronii*. Preliminary, stability studies of *Lactobacilli* sp. isolated from Kutajarista were compared to commercial *Lactobacillus rhamnosus* strain LGG in Kutajarista matrix. *Lactobacillus plantarum* strain from Kutajarista was found to be stable for longer duration (≈14 days) in comparison to commercial strain (≈8 days). *Saccharomyces cerevisiae* strain isolated from these ferementation processes could enhance the viability of *Lactobacilli* suggesting specific interaction between these strains which helps in increased survival in Kutajarista matrix.

Conclusions:

This study highlights the potential use of alternative sources like ayurvedic fermented products for isolation of indigenous probiotic microbes. Kutajarista has unique therapeutic properties for gastrointestinal disorders and therefore it could be an excellent vehicle for incorporation of these strains. In future, it may also help in formulating herbal medicines in conjunction with probiotics, which are safe and are effective alternative to antibiotics.

Potentiality of two promising putative indigenous strains of probiotic lactobacilli for boosting gut health and immunity

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Introduction:

Lactobacilli constitute an important component of the commensal gut microflora in both human and animals and are acclaimed as the key member of probiotics. Since probiotic lactobacilli express a multitude of general and specific health promoting functions, there is considerable scope to explore probiotics in boosting gut health and immunity besides playing a crucial role in managing inflammation related disorders such as IBD, Ulcerative colitis, Traveler's diarrhea, Crohn's disease, etc. However, the mechanisms by which these non-pathogenic micro-organisms exert a positive influence upon ingestion largely remain unclear at molecular level. Quantification of genes encoding specific physiological attributes at transcriptional level is an important criteria to know gene functionality

Methods and Results:

Initially, seven putative indigenous probiotic *Lactobacillus* isolates i.e. *L. plantarum* Lp9, Lp72, Lp75, Lp77, Lp90, Lp91 and *L. delbrueckii* subsp. *bulgaricus* CH4 along with a reference culture *L.plantarum* CSCC5276 were screened based on their hydrophobicity and cell adhesion property on HT-29 and Caco-2 cell lines. Lp9 and Lp91 showed maximum hydrophobicity and adhesion scores and therefore, were selected for detailed studies on quantification of relative expressionof the genes involved in acid and bile tolerance along with colonization under *in vitro* conditions simulating gut environment as well as their immuno-modulatory function in HT-29 cells by RT-qPCR. Amongst the two, Lp91 exhibited relatively higher acid tolerance, as revealed by 4.7-fold up-regulation of the '*atp*D' gene as well as higher survivability at pH 2.5 after 90 min.

Expression of '*bsh*' gene was up-regulated optimally in all the selected strains under 2% bile concentration with maximum attained with Lp91. In the context of surface proteins playing an important role in bacterial adherence, '*mub*' gene was found to be maximally expressed (20.07 ± 1.28) in Lp9 in MRS broth containing mucin (0.05%) with bile and pancreatin at 1% each (pH 6.5). Almost the same trend (30.92 ± 0.51) was recorded in the expression of '*map*A'

in Lp9 under same set of conditions. 'EF-Tu' gene, on the other hand, was expressed highest in Lp91 only in medium with 0.05% mucin. However, there were inter strain variations in respect of the expression of all the three surface proteins. All the probiotic cultures were able to exhibit immuno-modulatory properties by up regulating MUC2 expression and the keyanti-inflammatory cytokines (IL-10, IFN-α and TGF-β) and regulating the pro-inflammatory markers (IL-8, TNF-α, IFN-γ and IL12p35) and other signaling molecules (COX-1, COX-2 and Hsp70). Maximal 'MUC2' gene expression in HT-29 cells was recorded with Lp5276 closely followed by Lp91 under both pre and co-culture treatments with probiotics and LPS. Lp91was rated as the most effective by significantly up regulating IL-10 and IFN-α expression in HT-29 cells under pre-culturing conditions. However, Lp9 was the most potent by evoking the maximal expression in HT-29 cells under co-culture conditions. Lp91 was also found to be the most effective probiotic as immunosuppressant as it was able to significantly down regulate the expression of all the four cytokines i.e. IL-8, TNF-α, IL12p35 and IFN-γ in HT-29 cells under both the conditions.

Conclusion:

Lp91 and Lp9 were the most potent indigenous probiotic *Lactobacillus plantarum* strains based on their high hydrophobicity, acid, bile tolerance and adherence property and expressing high levels of surface proteins. Besides, they also exhibited strong immuno-modualtory properties in HT-29 cells and mucosal barrier function. These strains hence can be explored as prospective biotherapeutics in the management of gut related diseases.

Safety investigation of probiotic strain *Lactobacillus helveticus* MTCC 5463 using complete genome sequence annotation studies

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Introduction/Significance of the problem:

We are in an era of science-based risk assessment process that forms the rationale of assessing the safety of food which also percolates to selection of probiotics. The wellness seeking populationvare walking the tight rope between being a sceptic or convert and demand "proof of concept" to become the latter. Today a GRAS status is not enough to vouch for the safety of a probiotic strain. Complete genome sequencing and functional genomics provide meaningful insight on its biosafety. Our isolate *L. helveticus* MTCC 5463 is known to exhibit significant antimicrobial activity, reduce cholesterol levels in humans and elicit positive immunomodulating effects. We performed a comprehensive safety assessment of a MTCC 5463 based on the whole genome sequence. The key safety aspects include antibiotic resistance, production of harmful metabolites, potential for virulence, biogenic amines, D-lactic acid, azoreductases and nitroreductases. This approach can provide a deep safety investigation of novel probiotic strains.

Methods and Results:

Genetic confirmation of the pure culture was done by 16S rRNA universal primer based PCR followed by cycle sequencing and BLAST. De Novo sequencing of MTCC 5463 was done using 454 GS FLX Titanium instrument (Roche Applied Sciences) and assembling of contigs using GS Assembler Software (GenBank accession numbers AEYL01000001 to AEYL01000593.).The genomic library contained 119,569 reads, and assembly generated a 1,911,350-bp single chromosome. Gene annotations were done using MG-RAST contributing to the desirable traits of a probiotic strain, the genome totally lacks the presence of mobile genetic elements like plasmids, transposable elements and gene transfer agents suggesting that transfer or acquisition of other antibiotic resistances are highly improbable in this strain. Absence of complete prophages and scarcity of IS element play important roles for industrial applications and in promoting genome stability. The subsystem analysis revealed that MTCC 5463 bears 26 genes for virulence, disease and defense. Of them 25 are assigned to resistance to antibiotics and toxic compounds which include copper homeostasis (3), cobaltzinc-cadmium resistance (3) and mercuric reductase (4). Homologs of the antibiotic resistance determinants found in the genome of MTCC 5463 include one fragment on streptothrin resistance, two putative genes for tetracycline resistance and ribosome protection type, five putative genes for flouroquinolone resistance, two genes for beta lactamase and three putativemultidrug resistance efflux pumps. However, a greater threat associated with chromosomallyencoded resistance mechanisms is the potential for high-level resistance to emerge during the course of therapy especially in the presence of multidrug efflux pump genes. For safety evaluations, it is important to determine the location and nature of beta-lactam, flouroquinolone and tetracycline, resistance determinants and the route as in acquired or intrinsic.

Conclusion:

Bioinformatic assessment of biosafety of probiotic strain MTCC 5463 provides comprehensive knowledge on its genomic stability, potential of antibiotic resistance, virulence and production of harmful metabololites. Addressing the concern over antibiotic resistance transfer to the gut microbiota, the absence of mobile genetic elements and chromosomally-encoded antibiotic resistance mechanisms satisfies the queries in relation to its pathogenicity, toxigenicity, allergenicity and biosafety.

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Shree Additives Pharma & Foods Ltd-A Profile

One of the leading manufacturers of food ingredients, servicing the food industry since the last two decades.

We are manufacturers of SAVOURY SEASONINGS, DAIRY INGREDIENTS, VITAMIN & MINERAL PREMIXES, FLAVOURS & BATTERS FOR COATING.

Dairy Ingredients comprise **fruit preparations, flavours, flavoured milk improvers and seasonings** to enhance the taste, texture and appearance of dairy products Our product range:-

Fruit Preparations are available for yoghurts, yoghurt drinks, ice-creams and pie fillings. Product range includes Mango, Orange, Lemon, Strawberry, Pineapple, Tropical Mix Fruit

Seasonings are available for paneer, buttermilk, cheese spread and processed cheese. Product range includes Jeera, Pudina, Spicy and Garlic for buttermilk & Black Pepper, Coriander, Pasrley and Red Chilly for paneer and cheese.

Flavours are available for Ice-cream, Yogurt, Lassi, Flavoured Milk, Flavoured Milk Improver, Whey Drink

Product range includes Kesar Elaichi, Strawberry, Vanilla, Chocolate, Thandai and many more.

Our company is an IS0 22000:2005 certified. We have been awarded a NSIC-CRISIL rating of SE 1B indicating highest performance capability. We also have a state of the art testing laboratory for quality analysis and quality assurance.

Product Customization is our USP.

We have the facilities for new product development and we can provide individually adjusted solutions for any product as customer's demand. Please contact us for any requirements.

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Poster Awardees

Screening of indigenous probiotic strains for their role in the generation of regulatory responses and study of the underlying mechanisms of probiotic-induced mucosal tolerogenicity

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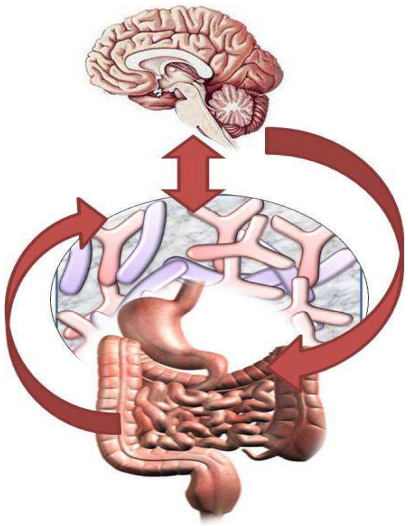
Gut microflora contains a substantial number of gram-positive probiotic bacteria, which are far from inert commensals. Instead, they are involved in strengthening of the immune system by providing critical regulatory signals through Toll-like and NOD-like receptors (TLRs and NLRs) expressed on the epithelial and immune cells. One of the most promising areas of development in the field of human nutrition over the last two decades has been the use of probiotics and recognition of their role in human health and disease. Recent studies suggest that probiotic-induced immune response not only depends on the bacterial species, but also on the specific strains. Hence, screening of new indigenous probiotics and detail study of the underlying mechanisms of their beneficial role will significantly contribute to promote health and prevent/treat diseases. Isolation and validation of indigenous *Lactobacillus* strains as potent probiotics were performed following ICMR-DBT guidelines. Indigenous probiotic strains were screened for their anti-inflammatory functions and some strains showed comparable suppression of NF-kB-mediated transactivation as the reference *Lactobacilli*. These organisms were also screened for their ability to produce regulatory response in the *in vitro* and *in vivo* experiments. Several strains enhanced the production of IL10 and TGFβ from the epithelial and dendritic cells (DCs). In addition, they significantly induced the generation of CD11c+CD103+ tolerogenic DCs and increased the differentiation of CD25+FoxP3+ regulatory T-cells. This study identifies indigenous probiotic bacteria that may be therapeutically useful against autoimmune and inflammatory disorders. Further studies will explore the underlying cellular and molecularmechanisms of probiotic-induced mucosal tolerogenic response.

Stress induced dysbiosis of Gut Microbiota



Expression analysis of *Lactobacillus* adhesins under mucin exposure and its role in adhesion of different *Lactobacillus* strains

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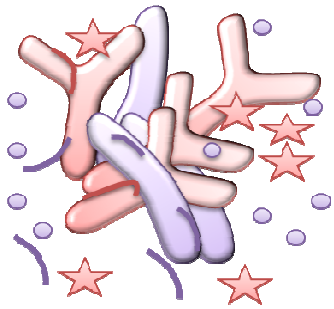
The adhesion of probiotic bacteria to the intestinal mucosa is a key process for colonization and persistence in the gastrointestinal tract. Several adhesion mediators have been identified in different *Lactobacillus* strains. The known adhesins - Elongation factor-Tu (EF-Tu) and mucus adhesion promoting protein (MapA) were amplified from the child fecal isolate *L. plantarum* CS24.2 and cloned into pET expression system. The proteins were purified with the Ni-affinity chromatography utilizing His-tag on expressed proteins. The purified proteins wereused toestablish the role of these proteins in the adhesion ability of different *Lactobacillus* strains using mucin and intestinal cell lines as an *in vitro* model for intestinal mucosa. Recently, the expression of these proteins has been reported to be up-regulated with the exposure of gastrointestinal environment in certain *Lactobacillus* strains. Thus, the second part of the study was to analyse the expression of EF-Tu and *map*A gene in *L. plantarum* strains using quantitative real time PCR. Understanding the role of EF-Tu and *map*A in adhesion of different *Lactobacillus* strains and expression analysis in presence of mucin would help in establishing these adhesion factors as marker for selecting adhesive probiotic strains.

Probiotic effector molecules for pharmocological application as Cost-effective and Feasible strategies



Whole genome sequence of two indigenous probiotic bacterial strains *Lactobacillus plantarum* Lp 91, *Lactobacillus fermentum* Lf-1 has been deciphered at Molecular biology Unit , NDRI,

Modulation of anti-inflammatory response in LPS stimulated cell line and mouse model with indigenous putative probiotic lactobacilli

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Action of probiotics on the host immune system has entered a new and fascinating phase of research in search for anti-inflammatory agents. The anti-inflammatory potential of indigenous probiotic *Lactobacillus* isolates was evaluated by reverse transcription - quantitative PCR (RTqPCR) in human acute monocytic leukemia (THP-1) cells. Further, the most potent and promising probiotic strain was selected for *in vivo* studies in Lipopolysaccharide (LPS) induced mouse model to determine the level of Vascular Cell Adhesion Molecule-1 ('VCAM-1'), expression of 'TNF-α', 'IL-6', Monocyte Chemotactic Protein-1 ('MCP-1'), Inter Cellular Adhesion Molecule ('ICAM-1') and 'E selectin' in aorta. Out of the 10 selected indigenous culture probiotic strain *Lactobacillus plantarum* Lp91 showed a significant ($P<0.001$) down regulation of 'TNF-α' by -1.45 fold relative to the control in THP-1 cells. All the strains evoked significant level of up regulation($P<0.001$) of 'IL-6' in THP-1 cells. Lp91 was selected for studying the relative expression of various proinflammatory cytokines, chemokine group fed with Lp 91 for 10 days, 'MCP-1', 'VCAM-1', 'ICAM -1' and 'E selectin' expressions'TNF -α' in THP-1 cells. In LPS challenged (2 h) mice and adhesion molecules in an *in vivo* based on its ability to down regulate LPS mice model were significantly reduced to 4.219, -3.135, 2.279 and 5.714 fold as relative to control conditions. Based on the results of this study, it can be concluded that Lp91 could serve as a potential candidate probiotic strain for further studies to explore it as possible biotherapeutic agent against inflammatory metabolic diseases including CVD.

Can metabolites of probiotics ease Behavioural Psychology: benefits beyond gastro-intestinal disorders?

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Psychiatric disorders which are on the increase globally, already rank among the leading causes of disability, and are expected to take over first place within the next few years. Probiotics, beneficial microorganisms are known to synthesize neuroactive compounds that affect the host's gastrointestinal and psychological health. Once ingested, probiotics enter an interactive environment encompassing microbiological, immunological, and neurophysiological components. The range of neurochemicals produced by probiotics includes neurochemicals for which receptor-based targets on immune and neuronal elements have been well characterized. The fermentative production of important neurochemical, GABA by probiotics has attracted greater attention. The development of traditional fermented products and newer therapeutic formulations with GABA are being viewed as channels for treating some of the neuropsychological disorders. Screening studies indicated the potential of tested LAB cultures to produce GABA. Selected culture, *L. bulgaricus* CFR 2028 produced highest yields (22.7 mM). Instrumental characterization by HPLC and structural characterization by MS confirmed extracellular production of GABA. Among the five different media tested, highest production of GABA was in the TYG medium, clearly indicating that the composition of the medium has a significant effect on the yield of GABA thus opening up avenues for the optimization of the medium components for maximization of yields. A time course study on the effect of substrate in TYG medium indicated that 2% MSG supported higher yields of GABA.

Facts On safety associated with probiotic consumption

- Lactobacillus infection estimated at 1 per 10^7
- Risk of lactobacillemia less than 1 per 10^6
- Impressive safety record in immunocompromised patients
- Low (not zero) opportunistic pathogenicity

Microbial word on Social Network

The Gut Microbiota & Health Section of the European Society of Neurogastroenterology and Motility launched an online Facebook platform titled "Gut Microbiota World Watch." for spreading knowledge and raising recognition of the importance of the gut microbiota for overall health.

https://www.facebook.com/GutMicrobiotaWW?

Major Recommendations of PAi Conference

The following recommendations emerged from the outcome of the various technical sessions in 1st Annual Conference of PAi and the International Symposium on 'Probiotics for human health – New Innovations and Emerging Trends' held on 27th -28th Aug., 2012 at IHC, New Delhi. These recommendations were the outcome of extensive deliberations made in the panel discussions based on the key issues which figured prominently during the presentations of invited speakers in various technical sessions.

Rich microbial diversity of Indian gut needs to be thoroughly investigated in search of novel probiotics due to different food habits, geographical, cultural and anthropological differences and the ethnicity. For achieving this long term objective, it is high time that an all India research coordinated project on these lines should be initiated on priority by identifying different groups from different geographical locations in the country through networking.

Since novel physiological function of probiotics are highly strain specific, it is very crucial that the probiotic organisms must be identified accurately at strain level by using advanced molecular techniques which can discriminate between the genome of closely related species and strains of the same probiotic genera without any ambiguity. In this context, whole genome sequencing of the probiotic strain is now recognized as the most authentic gold standard for probiotic identity.

Although, dairy based foods are considered as the best carriers of probiotics to confer their best health promoting functions in the consumers, there is a need to explore other food formulation also for probiotic supplementation to demonstrate their health efficacy. Traditional fermented foods which are already very popular and acceptable amongst the various ethnic groups in the country could be the most attractive targets that can be explored for value addition with proven probiotic strains.

Multicentric well-designed placebo controlled double blind clinical studies on the target local population should be conducted at least at two different locations to establish the functional efficacy of novel probiotic strains or food formulations developed with the same for a specific health claim.

The PAI family (Member details) after the release of 2nd issue March, 2012

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