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From Editor's desk

Dear Esteemed Members,

Warm greetings!

It gives us immense pleasure to launch the 14th issue of our most popular PAi Newsletter. This issue dedicatedly highlights 4th Biennial Conference of PAi and International Symposium on "Probiotic Therapy: Translating to Health and Clinical Practice" that was organized on 16-17th Feb 2018 at All India Institute of Medical Sciences (AIIMS), New Delhi, India, by Probiotic Association of India (PAi) in collaboration with American Society of Microbiology (ASM) and AIIMS, New Delhi. PAi would like to profusely thank all those who attended the conference and were part of the conference to make it a grand success. Our special thanks to Dr. Rama Chaudhry and Dr. V.D.Bamola besides entire AIIMS team for organizing this grand show. I am sure all of you would like the contents of this issue of the PAi newsletter which will provide information of conference to our members who could not participate in this conference to have a feel of the event. The issue also provides the information on recommendations of General Body Meeting. We have included all the articles sent by the esteemed members and thank them for their contribution and express our gratitude to them. Each and every member of the association is very special and important for us. We always look forward to you all for receiving articles of general interest for consumers and society, brief research findings for scientific fraternity, launch of new probiotic formulations in India and any other useful information related to probiotics from all our members. We request our readers to give us valuable suggestions to further improve the quality of our newsletter. We are happy to announce that Probiotic Association of India in association with Gut Microbiota and Probiotic Science Foundation (India) will be celebrating Elie Metchnikoff's Birthday as Probiotic Day on 15th May. The details will be given on PAi website as soon as we finalize. Finally, I would like to thank Dr. Shalini Sehgal, Assoc.Editor, PAi Newsletter who has contributed dedicatedly to bring this 14th issue of PAi Newsletter. If anyone who deserves appreciation for bringing this issue, it is Shalini Sehgal!

Wishing all a very wonderful, pleasant and healthy time ahead!

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HIGHLIGHTS OF THE CONFERENCE

4th Biennial Conference of PAi and International Symposium on "Probiotic Therapy: Translating to Health and Clinical Practice" was organized on 16-17th Feb 2018 at All India Institute of Medical Sciences (AIIMS), New Delhi, India, by Probiotic Association of India (PAi) in collaboration with American Society of Microbiology (ASM) and AIIMS, New Delhi. The Organizing Secretary was Prof. Rama Chaudhry, AIIMS and Co- organizing Secretary, Dr. Sunita Grover, ICAR-NDRI. A countrywide enthusiastic response was received from all over India for the conference. A total of 175 candidates from seven states of India participated in the conference.

The conference was inaugurated by Dr. Randeep Guleria, Director, AIIMS New Delhi ,Sh. Pawan Kumar Agarwal, CEO, Food Safety and Standards Authority of India (**FSSAI**), Govt. of India in presence of Dr. Katoch , Chief Patron, PAi and Dr. A.K.Srivastava, President, PAi.

The scientific sessions of the event was initiated with inaugural lecture by Prof. Pinaki Panigrahi, Director, Center for Global Health and Development College of Public Health Nebraska Medical Center. Dr. Panigahi was also honored with **Life Time Achievement Award** for his significant contribution in the field of probiotics, published in Nature

The scientific sessions were focused on probiotic therapy with special emphasis on multiple domains of probiotics including evidence from clinical trials, Probiotics and Gut Microbiome in Human Health and Disease, Probiotics in Pediatrics, Probiotics and Antimicrobial Resistance, Microbiota interventions to promote health, Probiotics and Microbiome - Advancement in Research & Technologies and Probiotics Status and Regulatory Framework and Ethical Issues in Clinical Trial. The presentations were made by renowned speakers Prof. Mary Ellen Sanders from ISAPP, Prof. Dan Merenstein from ISAPP, Dr. Anders Henriksson, Prof. Sanjay Patole, Dr. Yogesh Shouche, Prof. M Balasubramanyam, Dr. Ramesh Visvanathan and Dr Samir Bakshi and many more.

EMINENT SPEAKERS AT THE CONFERENCE

The sessions were chaired by various eminent and renowned personalities like Dr. M. L. Madan, Dr. Katoch, Dr. R.R.B Singh, Dr. Chauhan who gave insights befitting their vast experience and expertise.

CHAIRPERSONS OF VARIOUS TECHNICAL SESSIONS GIVING THEIR VALUABLE INSIGHTS

The key note address was delivered by Prof. Mary Ellen Sanders on a very pertinent topic - Is it time for live cultures to be included in official dietary recommendations? Dr. Sanders emphasized that consumers and scientists seem to agree that dietary strategies to support gut microbiota are worthy of recognition. This interesting lecture was by followed by ten Oral presentations by Young Investigator award category researchers pertaining to various aspects of Probiotics. A total of 51 abstracts were received out of which, 10 abstracts were selected for oral and 39 for poster presentation. The first presentation was on the development of probiotics from aquatic bodies for enhanced productivity in fish farming by Mr. Rahul Singh, Lovely Professional University, Punjab followed by the one on *Lactobacillus* strains that attenuate the *Salmonella enterica* serovar *typhimuriu*m mediated pro-inflammatory response in mice models by Ms. Sanjolly Gupta , GADVASU), Ludhiana. Metagenomic insights into the gut microbiota of autistic and normal siblings in India was presented by Ms. S.V. Aparna, Kerala Veterinary and Animal Sciences University and Comparative Analysis of Predominating Gut Enterotypes in Children of Varying Nutritional Status by Mr. Chandrasekhar, ICAR- NDRI, Karnal. Mr. Bhima from Osmania University, Hyderabad highlighted the probiotic and nutritional potentials of toddy palm nectar (Neera). Ms Shikha Sharma, CSIR-Institute of Microbial Technology, Chandigarh made an impressive presentation on Phylogenomic insights into origin of genes and lineages in species *Lactobacillus reuteri.* Mr. Iqbal Bhat of ICAR-NDRI, Karnal presented on the protection and restitution of *E. coli* and LPS induced epithelial barrier dysfunction by probiotic *Lactobacillus fermentum* (MTCC 5898) under *in vitro* environment while presentation on the gastrointestinal survival, adhesion and immuno-modulatory potential of indigenous *Lactobacillus fermentum* of human origin was made by Projoyita Samanta, AIIMS, New Delhi. Ms. Divya showcased a study to assess human gut microbiota and their metabolic end products using 16SrDNA sequencing and short chain fatty acids analysis in Indian population. The last presentation was on Modification of microbial flora in bacterial vaginosis through probiotic supplementation in pregnant women by Ms. Vasundhara. The chairpersons were Prof. Panigarhi and Prof. Dan Meintesin who were highly appreciative, of all the ten presenters and Prof. Panigarhi offered to present Rs. 5000/- cash for all the ten participants. Three best oral presenters were honored with PAi Young Scientist Award and 6 poster presenters were awarded for Best poster award for their valuable findings.

LIST OF SELECTED ORAL PRESENTATORS (YIA)	
1. Arun Chauhan	Lovely Professional University, Ludhiana
2. Sanjolly Gupta	Guru Angad Dev Veterinary and Animal Sciences University (Gadvasu), Ludhiana
3. Aparna S V	Kerala Veterinary and Animal Sciences University, Kerala
4. Chandrasekhar B	ICAR -National Dairy Research Institute ,Karnal
5. B. Bhima	Osmania University,Hyderabad
6. Shikha Sharma	CSIR-Institute of Microbial Technology, Chandigarh
7. Mohd. Iqbal Bhat	ICAR -National Dairy Research Institute ,Karnal
8. Projoyita Samanta	Department of Microbiology, AIIMS, New Delhi
9. Divya Pandey	Department of Microbiology, Aiims, New Delhi
10. D. Vasundhara	National Institute of Nutrition, Hyderabad

YOUNG INVESTIGATOR AWARDEES -2018

1. Dr. Shikha Sharma
2. D. Vasundhara
3. Dr. Aparna S.V
4. Dr. Projoyita Samanta

POSTER PRESENTATION AWARDEES	
1. Tej Bahadur	Department of Microbiology, AIIMS, New Delhi
2. V D Bamola	Department of Microbiology, AIIMS, New Delhi
3. Archana Anthappagudem	Department of Microbiology, University College of Science, Osmania University,Hyderabad
4. Tasneem Ravat	Department of Foods, Nutrition and Dietetics, College of Home Science Nirmala Niketan,Mumbai
5. Shashank Singh	National Agri-Food Biotechnology Institute (NABI), S.A.S. Nagar, Punjab

A skit was also presented by a team of ICAR-National Dairy Research Institute Karnal, India, to spread the awareness message on AMR and Probiotics explaining the concept and application of probiotics in a simplified and artistic manner.

Another interesting event was also organized this time around -a painting competition on probiotics for school children. Eight paintings from four different schools were awarded for their colorful diagrammatic presentation of probiotics.

AWARDED PAINTINGS BY SCHOOL CHILDREN

The conference ended with panel discussion on regulations and the way forward .The members included Dr A K. Srivastava, Dr V K Batish, Dr. Sunita Grover, Prof. Rama Chaudhry, Dr Neerja Hajela, Prof. Panigarhi and Dr. Mary Ellen Sanders. The panel discussion was followed by award ceremony and vote of thanks delivered by Prof Rama Chaudhry., Organizing Secretary of the conference.

The General Body Meeting of the Probiotic Association of India was held on 17th February, 2018 after the conclusion of the 4th Biennial Conference at AIIMS, New Delhi. The meeting was presided over by Dr. A.K. Srivastava, President- PAi in the presence of the office bearers -Dr. V.K. Batish, Secretary and Dr. Sunita Grover, Treasurer. There was also the presence of Dr. R.R.B. Singh, Officiating Director, ICAR-NDRI. Dr. Srivastava welcomed all the members and invited Dr. Sunita Grover to present the financial statement and other agenda. She presented the expenditure statement of the financial years- 2015-2016 highlighting the finance details of 3rdBiennial Conference at NISER, Bhubaneswar, Odisha and the PAi Workshop held at Karnal in 2016-2017. Dr. Grover also explained the expenditure till date for the current financial year 2017-2018 Dr. Grover then presented the agenda items one by one for deliberations in the house.

- The revamping of the current PAi website for making it more dynamic which was duly approved by the house.
- Dr. Sunita Grover announced that there is a need to elect new office bearers which was reiterated by the President too. He agreed that the guidelines should be formalized for the tenure and job responsibilities.
- She also informed about the white paper about the protocols for QC of probiotics initiated by ILSI, India and informed that PAi would request them to proceed on this jointly. The contribution of the PAi needs to be duly acknowledged in the document and she agreed to pursue it further with ILSI, India.
- It was also announced that PAi will celebrate Metchnikoff Day this year in May with the support from Yakult, India.
- The President also proposed that National or Regional Seminars /Workshops should also be organized on annual basis across India which was approved by the house.
- The venue of the next i.e. 5th Biennial Conference was also discussed. Dr. A.K.Srivastava agreed to contact various organizations to host the next conference.
- Dr. Shalini Sehgal, Associate Professor in the Department of Food Technology of Bhaskaracharya College of Applied Sciences also proposed to form Delhi Chapter of the PAi to strengthen the activities of the association and increase its visibility among the stake holders. She stated that various other associations have gradually established their network across India with state wise chapters, making their respective

parent associations robust in term of members and reach. Dr. Srivastava agreed to look into the proposal after the formulation of the due guidelines for the same.

It was also decided to involve various stake holders in the activities of the organization especially the corporate members. The meeting ended with the vote of thanks.

SCHEDULING PROBIOTIC INTAKE FOR PRECLUDING ANTIBIOTIC ASSOCIATED DIARRHEA

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Healthy gut flora is associated with the overall fitness of an individual. Challenges in the normal rhythm of the body due to pathogenic microbes lead to infections. After receding the symptoms of a particular infection using antibiotic treatment, some individuals suffer from intense diarrhoea which is referred to as Antibiotic associated diarrhea (AAD). AAD usually develops after taking antibiotics followed by the disruption of normal gut flora, an unexplained diarrhoea or allowing the multiplication of *Clostridium difficile* and release of the toxins. Since the probiotic action is strain specific, Swajeswska *et al* in 2015 showed that *Lactobacillus rhamnosus* GG gave best results in prevention of antibiotic associated diarrhoea. For restoring the normal gut flora physicians recommend probiotic supplements as tablets or capsules along with antibiotics in order to restore the gut micorbiota. Viability of probiotics from these supplements during antibiotic intake should be maintained through careful use of probiotics. Probiotics and antibiotics should not be taken simultaneously. Probiotic tablets should be taken along with a meal or 30 minutes before a meal (Tompkins *et al.*, 2011). This will help these bacteria to multiply and start colonizing in the gut of humans. Followed by the resolution of infection using antibiotics, immense multiplication of *C. difficile* occurs and *C. difficile* diarrhoea (CDD) is the final outcome. *C. difficile* is an anaerobe which is resistant to routinely used antibiotics and shows sensitivity to vancomycin and metronidazole. According to a recent CDC report, people on or after antibiotics had a 7-10% increase in risk for *C. difficile* infection. Antibiotic stewardship and minimizing the complications of antibiotics using probiotics can reduce the likelihood of *C.difficile* diarrhoea.

Recent studies proved that probiotics like *Lactobacillus rhamnosus GG* and *Saccharomyces boulardii* were effective for remission of *C. difficile* diarrhea (Christine *et al.*, 2016). *Bifidobacterium* which is also a good probiotic as well as GRAS strain cannot be used in this condition because it shows sensitivity to vancomycin. Since, *Saccharomyces* can cause opportunistic infections in immunocompromised host, nowadays more studies are being directed on the consumption of *Lactobacilli*. *Lactobacillus* genus shows intrinsic resistance to vancomycin which is not transferrable and as such becomes a desirable choice for CDD in future. Preliminary trials using this probiotic venture is a success. For instance, *Lactobacillus reuteri* has also proved to have a positive influence against AAD (Kolodziej *et al.*, 2017). In nut shell, we can suggest that probiotics may be a future cure for these diarrhoeal diseases due to indiscriminate use of our wonder drugs. Go back to the past diet and say "NO" to the new diseases.

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PROBIOTICS IN NEONATAL SEPSIS

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A variety of microbes present throughout the human body have fundamental role in human health. Microbiome in every healthy individual differs remarkably by different microbes present in varied body parts such as skin, nasal cavity and vagina. *Actinobacteria, A. naeslundii, Cyanobacteria, Bifidobacterium* and *Lactobacillus* are some of the bacterial species commonly found in healthy human body. (Chua, 2017). Alterations as a result of dysbiosis in these microbes can result in many health problems and can be cured using probiotics. Probiotics strains are "Non-pathogenic microorganisms that confer a health benefit on the host when administered in adequate amounts by displacing the pathogenic bacteria". An ideal probiotic should be able to have beneficial effect on host organisms, should not be toxic to host, should be resistant to stomach acids and should adhere in epithelial cells even in pathogenic condition (Moni *et al*., 2014). Neonatal sepsis is a clinical syndrome of systemic illness in neonates accompanied by bacteraemia occurring during the neonatal period. Neonates can come in contact with bacteria during delivery, by mother's milk or by surrounding environment. In healthy neonates, *Bifidobacterium* and *Lactobacillus* are the two strains majorly found in gastrointestinal tract. Abnormal colonization of pathogenic bacteria like *Klebsiella*, *Enterobacter* and *Citrobacter* in GI tract can lead to neonatal sepsis. Probiotic strains used in marketed probiotics for neonatal sepsis include *L. acidophillus, L. fermentum, L. gasseri, L. reuteri, L. rhamnosus, L. plantarurm* and *L. brevis* (Moni *et al*., 2014).

Adhesion of probiotics to the intestinal mucosa is associated with several health effects of selected probiotics (Hanne Jensen, 2004). A desired feature in probiotic selection includes antipathogenic properties, survival of microbes when passing through GI tract (gastric acid and bile) and adherence to intestinal epithelial cells. *Lactobacillus* species are majorly present bacteria in healthy neonates and its screening is important. Different type of adhesion models like intestinal mucosa, cell culture, immobilized mucus are discovered to mimic *in vivo* conditions. Findings revealed that *L. reuteri* strains tolerate gastric and small intestinal conditions very well and have a high adhesion capacity making it the best probiotic strain (Hanne Jensen, 2004).

The most important application of probiotics is to prevent pathogen colonization and to stimulate the host immune response. Several studies have noted the effect of probiotics on various gastrointestinal and extra-intestinal disorders including prevention of diarrhoea, lactose intolerance, protection against intestinal infections and urinary tract infection (Nagpal et al., 2012). Eventually, the understanding of the action of probiotic microorganism may be a powerful tool to design new strategies for the prevention and treatment of specific human diseases.

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ISOLATES FROM HUMAN BREAST MILK AND *IN VITRO* BIOSAFETY ASSESSMENT

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Probiotics are live bacteria which when consumed in adequate amounts confer a health benefit on the host. Microbes used as probiotics are derived from different genera and species and have been studied for a variety of health and disease endpoints. Probiotics favourably alter the intestinal microflora balance, promote intestinal integrity and mobility, inhibit the growth of harmful bacteria and increase resistance to infection and should possess the properties like survival in the gastrointestinal (GI) tract, persistence in the host and proven safety for consumers. The survivability and colonization in the gastrointestinal tract are considered critical to ensure optimal functionality and expression of health promoting physiological functions by probiotics. Also the protective role of the probiotic bacteria against gastrointestinal pathogens and underlying mechanisms has received special attention as such interaction has served as one criterion for selecting new probiotics for human use. For colonization, they should exhibit good surface hydrophobicity and aggregation properties.

Human milk constitutes an interesting and important source for obtaining new and specific probiotics strains for neonates, aiming for proper development of the gut microbiota and the immune development in infants. Earlier, human breast milk was considered to be sterile, but recent studies have shown that human breast milk contains different species of good bacteria which shows certain health benefits to the infants. Our study was focused to isolate and characterize such potential probiotic strains from human breast milk and to assess their *in vitro* biosafety aspects. We could isolate a total of 114 bacterial strains from different milk samples and out of which seven isolates showed potent probiotic properties such as acid tolerance, bile tolerance, antimicrobial activity and antibiotic susceptibility. The probiotic properties of these isolates were also compared with respect to standard probiotic strain, *L. plantarum*.

However, mere assessment of probiotic properties of such bacteria is not adequate to comply them as probiotics. Assessment of biosafety must take into account the nature of the microbes being used, method of administration, level of exposure, health status of users and physiological functions they are called on to perform. Hence, these isolates were scrutinized for their biosafety aspects such as antibiotic resistance, production of biogenic amines, mucin degradation, hemolytic activity, gelatinase production, bile salt deconjugation, etc. Autoaggregation of probiotic strains appeared to be necessary for adhesion to intestinal epithelial cells and coaggregation abilities may form a barrier that prevents colonization by pathogenic microorganisms. From seven probiotic isolates, two isolates showed mucin degradation activity and two isolates were found to be hemolytic (β-hemolysis). Hence, from seven isolates, only three isolates were found to be safe on the basis of the above said assessments for biosafety. These isolates also showed good auto-aggregation property. The molecular identification of these isolates is being carried out. However, the biochemical characterization suggests that these isolates belong to genera: *Lactobacillus*, *Pediococcus* and *Leuconostoc*. Our study suggests that these probiotic bacteria may be useful and can be employed for human consumption after the *in vivo* assessment of biosafety aspects and clinical trials.

EFFICACY OF PROBIOTIC COMPOSITE SUBSTRATE ON MURINE FAECAL ENZYMES

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Colorectal cancer (CRC) is the third most common form of cancer and one of the major health problems in the world. Epidemiological studies indicate that the incidence of colon cancer in humans is associated with dietary habits- a high-fat and low fibre diet appears to constitute an elevated risk for colon cancer, as compared to a high-fibre and low-fat diet. Certain bacteria are associated in the colon to convert procarcinogens to carcinogens. Beta- glucuronide, β-glucosidase etc. are the bacterial enzymes that are implicated in the formation of mutagens, carcinogens and various tumor promoters. Most probiotic foods in the markets worldwide are milk-based, and very few attempts are made to develop probiotic foods with the use of other fermentation substrates such as composite substrate. The combination of cereals and milk will have a synergistic effect to provide better nutrition and will ultimately lead to a novel probiotic food product.

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. Probiotic organism *L. acidophilus* NCDC-13 (4% inoculum) was grown in the substrate at 37°C for 8 h and used for further study. Swiss albino male mice with an average weight of 17 to 18 g were kept in a well-ventilated room in the Small Animal House of the Institute (NDRI, Karnal), and were provided with water and synthetic diet. The effect of fermented substrate on activity of faecal enzyme β-glucuronidase (fβG) that promotes the conversion of pro-carcinogenes to potential carcinogens was studied as described by Marteau *et al.* (1990) with some modifications.

The quantities of p-nitrophenol liberated from p-nitrophenyl β-D-glucuronide (PNDG) by fβG being directly proportional to the absorbance and the activity of the enzyme was expressed in terms of the absorbanceThe data were compared between control (unfermented) and fermented (probiotic) group.

The influence of feeding probiotic and unfermented substrate to mice on activity of β-glucuronidase enzyme is depicted in Fig 1. The enzyme activity during the pre-feeding stage (Base Line 1) showed no definite trend of enzyme activity. During the feeding stage (base line 2) the enzyme activity (depicted by the absorbance value) decreased in both groups, the decrease being more prominent in the probiotic-fed group. Highly significant ($P<0.01$) reduction in the activity of enzyme was observed in probiotic group after 15 days feeding, ~ 18.5% and 42.9% respectively, in control and probiotic groups. In the third stage (Base line 3) discontinuation of feeding resulted in increase of enzyme activity in both groups, but increase being more prominent in the control group. The results of the present investigations suggest that the composite substrate could be used as a base material for the preparation of variety of cost-effective probiotic food products.

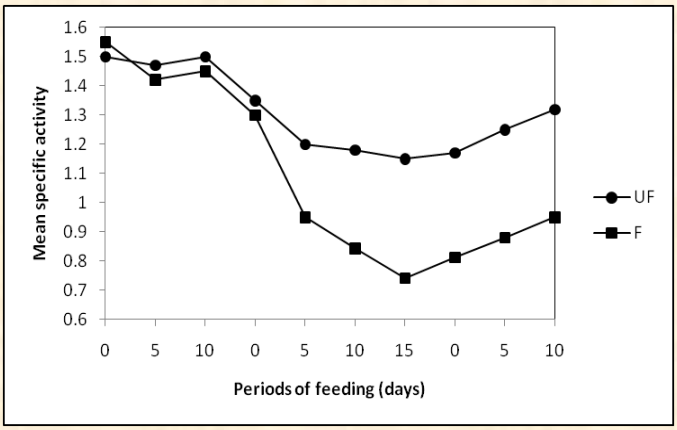

Fig. 1. Faecal β- glucuronidase activities for mice before feeding, during feeding and after the withdrawal of respective substrate. UF- unfermented substrate; F-fermented substrate, average of 6 mice and 3 trials; Base Line 1- pre-feeding period; base Line 2- Feeding period; Base Line 3- post-feeding period

Reference:

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NON-DAIRY PROBIOTIC BEVERAGES IN INDIA: CURRENT SCENARIO AND FUTURE SCOPE

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Probiotics are being sought after by consumers due to increase in health awareness. There is a steady shift towards preventive therapies amongst Indians which was not observed earlier. Availability of probiotics is also increasing due to expanding distribution channels across the country. All these factors collectively, widen the scope for non-dairy probiotic beverages in the Indian market. Currently, Indian popular probiotic brands from Mother Dairy, Amul, Yakult, Danone, Nestle etc are still essentially dairy based, however, brands such as Good belly probiotics, Cocobiotic, Pre probiotic enhancer etc. although have already ventured into the Indian probiotic markets but, are still in the nascent stage.

Fruits and vegetables are rich in phytochemicals which benefit human health in numerous ways by reducing oxidative stress and decreased risk of degenerative diseases. This makes them an ideal substrate for probiotics. While developing a functional probiotic food product, one of the important factor in is selecting a food system that is most suitable for the viability of a probiotic strain and also retains good sensory attributes, as the technological conditions later like heat or cell injuries due to osmotic stress might affect the probiotic cell viability. Recent researches are focused on development of alternates to dairy probiotics and fruit and vegetable juices have come up as a major ingredient of choice. It is due to the several advantages that they offer such as being a rich source of nutrients. Growth of probiotics is also supported by the high sugar content in fruit juices. Good flavours and refreshing nature also is an advantage and makes these juices suitable for consumption by various age groups.

There have been a significant number of recent advancements in the field of research regarding non-dairy probiotic beverages in India as well as across the globe. Kumar et al. conducted a study to find the suitability of various fruit juices in regard to *Lactobacillus casei.* They identified that the probiotic strain could survive in various fruit juices such as mango, sapota, cantaloupe and grape without the addition of extra nutrients. They also found the presence of antioxidants in these juices which are beneficial for the consumers (Kumar *et al*., 2013). Shah *et al.,* (2010) studied as to how the probiotic bacterial stability can be improved by using vitamins and antioxidants. A model fruit juice system with antioxidants such as extracts of green tea and grape seed along with vitamins was inoculated with strains of *L. rhamnosus*, *Bifidobacterium lactis* and *L.paracasei.* It was concluded that the ingredients with antioxidants promoted an environment that was favourable for the probiotic bacteria (Shah *et al.,* 2010)

Since the beverage market is moving towards healthier options there is an increased demand for functional beverages along with a need to explore non-dairy probiotic beverages in terms of factors that influence consumer acceptance as well as their optimal development as fruit and vegetable juices are already considered a healthy food product. However, further studies are required for testing new media, ingredients and processes to provide more options for the consumers.

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ROLE OF MICROBIOME IN PRE-TERM BIRTH: A DIAGNOSTIC AND A THERAPEUTIC OPPORTUNITY

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Preterm birth (PTB), or birth prior to 37 weeks of gestation, is a major cause of neonatal morbidity and mortality worldwide. As per the recent WHO report, more than 1 in 10 babies are born too early, globally. Approximately 1 million of infants die during their neonatal period, especially in low income settings. Survivors also face a lifetime of neurological impairment, because of sub-optimal use of technology in the care of such babies As per the WHO report, India ranked first with ~3.5 million preterm births annually.

PTB can be categorized into major subtypes including spontaneous PTB due to utero-placental ischemia and utero-placental haemorrhage (45%), preterm premature rupture of membranes (PPROM) (25%), or indicated PTB due to factors such as pre-eclampsia, eclampsia, or intra-uterine growth restriction (30%). Overall, risk factor may include, shortened cervical length, poly-hydramnios, low maternal body mass index, multiple gestation, systemic and genital tract infections (Hugh *et al.,* 2015; Alexis *et.al.,* 2017). Genital tract infection is associated with ~25-40% of PTB based on microbiological studies. The range may be larger but is limited by methods for detection of infection .Currently, only a few biochemical markers are available for the assessment of PTB (commercially available as PTB prediction kits). These include cervical IL-6, serum C-reactive protein (CRP), fetal fibronectin, β-hCG, and placental α-microblobulin (Mohammed *et.al.* 2017). However, these diagnostic kits have the disadvantages of high cost and limited accuracy (~60-80%) and high chances of false positive prediction. In addition, these kits only diagnose occurrence of PPROM but fail to predict PTB early or diagnose associated pathogen.Dysbiosis is the primary reason for increased chances of infections in the genital tract of pregnant females, which may lead to preterm delivery. Based on our meta-analysis of the genital tract microbiome and its relation to PTB, it is established that presence of *Lactobacillus* bacteria (e.g., *L. crispatus*,

L. iners, *L. jensenii*, *L. gasseri*) in the vaginal microbiome (Alexis et.al. 2017; Angela et.al 2016) prevents the overgrowth of other, more pathogenic microbiota by producing lactic acid and other bacteriostatic compounds. Importantly, in PTB cases, their numbers have been found to be significantly reduced (Romero et.al. 2014).

Presence of several pathogenic agents in the vaginal/placental/amniotic fluid microbiome have been found to be associated with preterm cases such as *Gardnerella*, *Ureaplasma*, *Mycoplasma*,*Staphylococcus*, *Streptococcus*, and *Escherichia coli* .With increasing number of studies related to microbiome and pregnancy, it provides a diagnostic opportunity to identify the presence of these pathogens early during the course of infection, hence, early prediction of PTB. In addition to treatment with appropriate antibiotics, another intervention is administration of probiotics. Probiotics are defined as live microorganisms which when administered in an adequate amount confer a health-benefit on the host. Probiotics offer multiple advantages such as immune-modulation, preservation of protective vaginal flora and elimination of infectious microbes. Hence, Probiotics, administered orally or vaginally constitute potential complementary therapy if combined with antibiotics for treatment of infections leading to PTB.

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UNDERNEATH THE UNCONVENTIONAL: PROBIOTICS AS AN ALTERNATIVE TO ANIMAL SACRIFICE IN EVALUATION OF ORAL COLON TARGETED DRUG DELIVERY

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Probiotics considered as unconventional nutraceuticals have found their application mainly as functional foods with a number of health benefits. Our group has worked upon an entirely new paradigm of probiotics i.e. as a surrogate to human colon milieu. To increase the therapeutic efficacy of drugs meant for colonic diseases like inflammatory bowel disease, colon cancer, Crohn's disease, ulcerative colitis, colonic infestations with worms, scientists have been working to develop formulations that precisely target the colon. A number of approaches have been tried to achieve this. These include time controlled, pH controlled, pressure controlled systems, azo-polymeric prodrugs and polysaccharide based formulations. So far, the polysaccharide based delivery systems have been considered as the most suitable techniques because of the unique trigger present only in colon i.e. the colonic microbiota. The anaerobic microbiota present in colon acts as a trigger to digest the polysaccharide coating of the drug for precise delivery to colon and facilitates its release therein.

The *in vitro* dissolution tests of solid oral dosage forms are officially recognized by the regulatory agencies as an essential quality control parameter. Selection of an appropriate media for these *in vitro* tests is crucial to their ability to predict the release of drug. The most commonly reported medium to evaluate oral colon targeted delivery systems is the one that utilizes colon contents of rodents. Apart from involving the sacrifice of animals, this medium has certain limitations like lack of reproducibility, high cost as well as need of expertise in dissection of animals. Other reported dissolution media include the use of human fecal slurries and goat cecal contents, which yield even less reproducible results. Our group has reported a unique, simple, inexpensive, and animal life sparing dissolution medium using probiotic culture.

Fluid thioglycollate medium containing probiotic mixture composed of *Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum, Bifidobacterium bifidum,* and *Saccharomyces boulardii* under anaerobic conditions is used as dissolution medium as surrogate to the colonic microbiota. We applied this medium for evaluation of different dosage forms including spheroids, mini matrix tablets, liquisolid tablets, and microspheres using different polysaccharides, i.e., guar gum, pectin, and xanthan gum. The results obtained were found to be comparable to those obtained using the conventional medium. Rather, dissolution profiles were found to be more reproducible. Our research group has, thus, established a suitable alternative for *in vitro* evaluation of polysaccharide based colon targeted delivery systems that eliminates the need for animal sacrifice.

Further Reading:

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PROBIOTICS SURFACE LAYER PROTEINS: A PROSPECTIVE PROBIOTIC EFFECTOR MOLECULES WITH METABIOTIC PROPERTIES

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The past decade has revolutionized the concept of probiotics as one of the most relevant tools to modulate gut microbiome for the human health benefits. Though the impressive potentiality of these microorganism has been envisaged profusely but certain ambiguity and risks are associated with their utilization due to their multiple mechanism of action which are dependent on specificity of strain and species utilized. Therefore, new alternative approaches are continuously being explored by keeping the view of retaining all the beneficial credentials of probiotics as beneficial microbes. Among these, the exploration of metabolic signatures of probiotics more precisely designated as “metabiotics” are now-a-days in limelight. They are low molecular weight (LMW) molecules having versatile chemical attributes and having the capability of interacting with surrounding environment and controlling the genes for various genetic, biochemical and physiological functions for maintaining the homeostasis of the cell. Among the wide arena of probiotic metabolites, surface layer proteins ubiquitously found in Gram-positive, Gram negative bacteria and in Archaea. They possess the property of self-assembly that leads to the formation of two-dimensional (2D) crystalline arrays on the surface of the cell. Research studies confirmed their regulatory role for controlling various signaling pathways and cellular process. The diversified function of surface layer proteins is dependent on different lattice structure present in different bacterial species. The functions may include as maintenance of integrity of cell membrane, protection against pathogens, lytic enzymes, mediator for adhesion to gastrointestinal tract, effective platform for ion-transport, resistance towards pH changes.

Though the surface layer proteins of pathogenic bacteria play pivotal role in their proliferation in the host cells and imparting the virulence properties but the surface layer proteins of probiotic can be considered as metabiotic component owing to their contribution in health benefits. Currently, various scientific studies reported beneficial role of the slps of various probiotic species. The purified slp from *Lactobacillus acidophilus* ATCC 4356 demonstrated the antimicrobial activity against *Salmonella enterica serovar Newport* in combination with nisin. More recently, the synergistic effect of nisin and SlpB, a surface layer protein isolated from *Lactobacillus crispatus* has potential to inhibit the growth of *Staphylococcus saprophyticus*. Research studies also confirmed the anti-tumor potential of surface layer proteins (12 and 15kDa) from *Lactobacillus plantarum L67* for the programmed cell death in HT-29 cells. The same protein has also been observed for the protection against the toxicity of cadmium chloride. Besides that, surface layer proteins have been immensely used for the supramolecular engineering for creation of biomimetic materials well as in nanobiotechnological applications. Therefore, it is conceivable to envision that the therapeutic and preventive avenues of surface layer proteins will be unravelled through comprehensive understanding.

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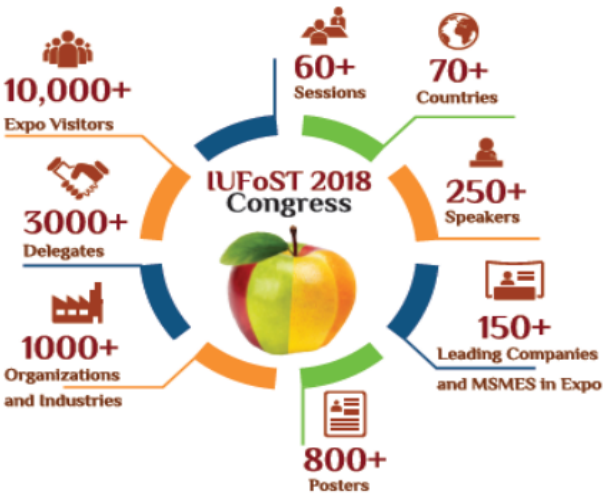

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