

# Probiotic Association of India

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#### Dear All

This is to inform all the members of PAI & others that 1st Annual Conference of PAI and Symposium "Probiotics for Human Health - New innovations and Emerging Trends" will now be held on 27th-28th August, 2012 at India Habitat Centre, New Delhi.

V.K.Batish

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### From Editor's desk

Warm greetings to our viewers on the eve of launch of 2<sup>nd</sup> electronic issue of **Probiotic** Newsletter. As promised in our inaugural issue, this special issue has been dedicated mostly to the invited contributions from the registered members of Probiotic Association of India from different parts of the country. Overall, the response has been quite overwhelming and we appreciate the interest shown by the enthusiastic members particularly the students and young researchers from the faculty who are very keen to share their knowledge and expertise in probiotics with the readers/viewers regularly visiting our web site. Since, PAI family is now expanding exponentially, we expect greater participation from our esteemed members in further improving the quality of forthcoming issues of Probiotic Newsletter. We look forward to their inputs in the form of clippings of the new breakthroughs in probiotic research and articles of general interest to create awareness on the

immense potentials of probiotics and probiotic foods/formulations and their application in human health amongst the common public in the country. Your valuable suggestions will always inspire us to take probiotic movement to every house in the country that will eventually help in translating our dream of building a healthy and disease free society by promoting the consumption of at least a billion probiotics a day to keep doctor away. The editorial board desires that all the PAI corporate members also send their valuable inputs for the Probiotic Newsletter to enlighten all the stakeholders to the current happenings related to probiotics around the world from industrial perspective. Furthermore, we are again requesting our corporate members to provide their company profile for the Newsletter and PAI website. This time, the company profile of Chr-Hansen has been provided in the Newsletter alongwith the linkage in the PAI Website. Wishing you all a happy and quality healthy time ahead!

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#### Do probiotics combat ageing?

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Ageing is a complex biological process, which leads to gradual loss of capability of an individual to maintain homeostasis. In almost all mammals, antioxidant functions decline during ageing. Implication of reactive oxygen species (ROS), such as hydrogen peroxide and superoxide anions, which are

produced as by-products in aerobic organisms in gut and also by ultraviolet light, ionizing radiation, chemical reaction, metabolic processes and so on disturbs the cellular processes such as mutagenesis, carcinogenesis and ageing. Protection against oxidative stress in cells may be mediated by the antioxidant effects of compounds such as carotenoids. To protect cells against oxidative damage by oxidants, an antioxidant system has apparently got evolved in aerobic organisms. Antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), etc., constitute an important defense system to invade ROS in in vivo. Most of the organisms possess antioxidant defense and repair systems that have evolved to protect them against oxidative damage. Hence, keeping this background in mind, the evaluation of the antioxidant properties of specific chemical scavengers could be extremely valuable for their potential use

Providing the beneficial probiotics can prevent oxidative stress by producing antioxidant metabolites which in turn prevent premature ageing and provides healthy life

in preventing or limiting the damage induced by free radicals. Accordingly, various naturally occurring substances as well as biotechnological products are receiving continuous attention from the viewpoint of antioxidation. Even though, various compounds have proved to be quite effective antioxidants in biological systems, they are not recognized by authorized organization due to food applications. Daily food practices may also participate in the process of ageing. The imbalance in the antioxidants can be controlled by providing functional foods carrying probiotic microorganisms such as Lactobacillus and Bifidobacterium species as supplements. Providing the beneficial probiotics in the foods can also prevent oxidative stress by producing antioxidant metabolites which in turn prevent premature ageing and provides healthy life. In the search for new approach in recent years, researchers are focusing on the screening of antioxidants from probiotic lactic acid bacteria, a prospective application for food and pharmaceutical industry. The concept sounds challenging, as there is lack of adequate published reports to support these health claims.

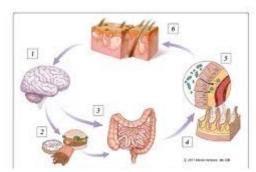
#### Can probiotics influence our minds?

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Food products containing probiotic bacteria have been known for centuries for their incredible benefits in digestion and gut health, a fact that has been supported by research. But very few could perceive that probiotics can also benefit the brain? Until the 21st century, the concept that probiotic bacteria

such as LAB administered to the gut could influence the brain seems like stuff taken straight out of science fiction. The latest research findings in the area indicate that probiotics' realm of influence is not just confined to the gut but goes beyond it, including brain health. This new found interaction between the gut and brain health has been termed gut-brain axis, and has generated lot of interest and huge excitement among the researchers in the field of probiotics. The gut-brain axis shows the mechanism by which the "end organ", the intestine is regulated by the enteric nervous system which is linked to the brain via the autonomous nervous system. The brain connects to the autono-

The autonomous nervous system is linked to the gat by the enteric nervous system, which affects the intestines and in turn, the intestines influence the brain.



mous nervous system which regulates involuntary functions such as heart rate, digestion,

etc. The autonomous nervous system is linked to the gut by the enteric nervous system, which affects the intestine and in turn, the intestine influence the brain. The concept is strengthened by reports revealing that probiotics are capable of secreting neurochemicals/transmitters (e.g. serotonin) that interact with the brain via the connections mentioned above. Certainly, there is more that meets the eye and the evidence points to a vital connection between the gut microbes and the nervous system. The findings have opened a new field of microbial endocrinology where microbiology meets neurological science, thereby, further widening the scope and domain of probiotic applications for human health.

#### Food-grade lactics and Bacillus as probiotics in functional foods

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In order to develop novel native probiotic cultures from fermented foods, an innovative methodology was followed for screening, identification and

the delivery of these bacteria for the local population. Initially, acid and bile stress tolerating bacteriocinogenic LAB were isolated from soy curd (Okra sample) residue for probiotic application. Lactic cultures, *Pediococcus pentosaceus* OK2, OK3, OK28 were found to have appreciable probiotic properties compared to other cultures. In addition, a large number of bacteriocinogenic LAB isolated from idli batter and milk were evaluated for probiotic properties. Initial selection was based on antimicrobial activity against food-borne pathogens and the selected isolates were further tested for probiotic properties. Commercial pharmaceutically applicable cultures which include *Lactobacillus rhamnosus* GG, *Enterococcus faecium* DPC1146 and *Enterococcus faecium* MTCC5153 an intestinal isolate producing enterocin-A were investigated as reference strains for comparison. Among the test isolates, *Pediococcus* spp Cu5 of curd origin demonstrated good probiotic property. Furthermore, three potent phytate degrading LAB with probiotic properties were also assessed for functional food formulation. Since, safety evaluation of the probiotic cultures is mandatory, one of the pediocin PA-1 producing strain of *Enterococcus fae-*



cium Acr4 was evaluated in vivo by acute toxicity study using Wister rats. Upon feeding live cells, there was no mortality or any toxicological changes in the animals. No observed behavioral changes were recorded in probiotic fed rats. Upon examination of faeces for microbiological counts, viable cells of the culture were recorded by plate count and their authenticity was confirmed by ped B PCR. Hematological studies did not show any significant difference compared to control. Histopathology of vital organs was also normal.

Determining the antibiotic resistance in probiotic bacteria and starter culture is vital to ensure that they do not transfer such traits to other bacteria. In this regard, we have studied co-existence of erythromycin and tetracycline resistance genes in LAB isolated from naturally fermented foods. Several of these cultures isolated and characterized for probiotic applications can be delivered through fermented foods. Attempts were also made to explore selected probiotic strains in the development and characterization of probiotic food gel. In this context, a method for producing encapsulated microspheres and freeze dried probiotics using food grade polymers was developed. *Lactobacillus casei* strain Shirota, *L. rhamnosus* GG and *Enterococcus faecium* MTCC 5153 were microencapsulated using different food-grade hydrocolloids and analyzed for probiotic properties in comparison to free cells. Recently, we have also characterized probiotic properties of native bacteriocin producing *Bacillus* cultures and compared the results with *B.subtilis*168 and *B. coagulans*. All the test strains were found to grow in the presence of 8% bile concentration and at alkaline pH (8 & 10). When compared to *B. coagulans*, few isolates showed better growth in the presence of acid (pH2). All the isolates showed potential scavenging activity and were capable of reducing serum cholesterol which are additional properties of probiotic bacteria.

# Development of probiotic low fat cheddar cheese with exopolysaccharide producing probiotic *Lactobacillus fermentum* CFR2195: textural and rheological effects.

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Fat reduction and low moisture content in low fat cheddar cheese is associated with many textural and functional defects. In our study, we have



used probiotic *Lactobacillus fermentum* CFR 2195, a human driven Lactic acid bacterial (LAB) isolate to improve rheological and textural characteristics of reduced fat cheddar cheese. The Exopolysaccharide (EPS) producing probiotic culture (8%v/v) was used for formulation of reduced fat probiotic cheddar cheese. All samples were stored in ripening room at 4°C for 15 days. The effect was studied on texture and rheological properties of reduced fat cheddar cheese. The hardness, gumminess and chewiness of EPS cheese were found to be 25.5 N, 9.26 N and 5.1 kgf, respectively which were comparatively lesser than those recorded with the control cheese where the corresponding values were as 33 N, 11.2 N, 6.8 kgf indicating that EPS cheese samples were significantly more softer and peliable than the control cheese. The change in values and slopes of viscoelastic moduli G′, G″ and G\* as a function of frequency were found more consistent ≈2×10<sup>4</sup> Pa in EPS cheese and thus indicate less frequency dependence and less deformation. The softer texture, better flow, less deformation may be attributed to water stabilization ability of EPS. Since the strain has already been proven as the potent probiotic, the cheese can also be used as carrier of probiotics to strengthen the hu-

man colonial health. Hence, the study concludes that *L. fermentum* CFR 2195 can be used as probiotic adjunct culture in the low fat cheddar cheese industry to provide soft and better flavored reduced fat cheddar cheese with human health benefits.



#### Probiotics - The incredible microwarriors for fighting colon cancer

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Colorectal cancer or rectal cancer is ranked as the third most common form of cancer as per the latest available stats from the World Health Organization. Colorectal cancer (CRC) accounts for 655,000 deaths each year. Everything about our modern lifestyle – stress, smoking, obe-

sity, sedentary living – increases our risk of acquiring various cancers. The gastrointestinal tract functions like internal skin, but it has about 150 times more surface area than our outside skin. It has been thought for quite some time that the gut microflora could be involved in causing CRC. Beneficial bacteria may prevent the growth of these organisms, and even produce anticarcinogenic substances. Since probiotics modify the balance of gut bacteria in favor of beneficial bacteria, it appears more than a possibility that probiotics might help in reducing the CRC risk. Probiotics bacteria being very active in the colon, compete with harmful bacteria for food and space and thus are helpful to suppress them.

Epidemiological studies show a link between CRC risk and high fat diets and this is thought to be due to raised levels of bile acids (which help digest the fat) in the colon. The breakdown

Everything about our modern lifestyle - stress, smoking, obesity, sedentary living - increases our risk of acquring various cancers

products of bile may have a cytotoxic effect on the cells lining the colon, increasing cell proliferation and possibly cancer. Modulation of the intestinal microflora through probiotic interventions may downregulate the activity of one of the enzymes ( $7\alpha$ -dehydroxylase) involved in the formation of these toxic products. Additionally, probiotics may also reduce the toxicity of bile salts by binding to them.

To summarize, probiotics may help by: Suppressing the activities of enzymes associated with carcinogen formation (e.g. β-glucuronidase, nitroreductase etc.), binding to mutagens, stimulating the activity of beneficial enzymes that inactivate carcinogens (e.g. glutathione S transferase), forming conjugated linoleic acid (CLA), a compound with anti-inflammatory properties.

# Effect of different process variables on chemical attributes of plain yoghurt and its feeding impact on albino rats

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Yoghurt is a probiotic product obtained by fermentation of milk using a pure starter culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Plain yoghurt was prepared from fresh cow (M1) and buffalo (M2) milk standardized at three levels of fat 4% (F1), 5% (F2) and 6% (F3); three levels of SNF 10% (S1), 11% (S2) and 12% (S3) along with 5 levels of starter culture of *Streptococcus salivarious subsp. thermophilus* (S) and *Lactobacillus delbruechii subsp. bulgaricus* (*L*) in the ratio of 1:1 (Set1), 1:2 (Set2), 1:3 (Set3), 2:1 (Set4) and 3:1 (Set5) at 2 temperature levels 39°C (T1) and 39°C (T2). Fat and SNF levels were adjusted in the milk using skim milk and fresh milk fat. Before adding starters, cultured milk was heated at 90°C for 10 minutes and cooled at and 40°C. The starter culture mix at 2% level, was added in each milk samples maintained at respective incubation temperatures for 6 hours. It was, therefore, thought prudent to select some factors and

Hoghart is a probiotic product obtained by fermentation of milk asing a pure starter culture of Lactobacillus bulgaricus and Streptococcus thermophilus.

work out their interaction effects on the quality of yoghurt. These scores decreased significantly (P<0.01) when the level of *S. thermophilus* and *L. bulgaricus* used for yoghurt was 3:1 due to increased acidity and reduced pH. It reduces the level of cholesterol which can be good supplement in the diet for the person suffering with hypercholesterolemia.



#### Present Status and Future Potential for Probiotic Dairy Foods in India

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Emerging research has described sophisticated mechanisms of communication between microbes and their host. Probiotics have the potential to impact physiological and metabolic parameters such as immune system modulation, gut barrier function, level of metabolic end products available to serve as substrate for epithelial cells, binding or degradation of potential carcinogens, enzymatic alteration of bile acids and competitive exclusion which are also influenced by normal commensal microbes.

#### Status of probiotics in India

Currently, some of the pharma preparations of probiotics are used as prescription drugs. The perception that fermented milk or dahi is beneficial has already been widespread across India because, traditionally, these products have been used since Vedic times for the treatment of diverse conditions such as skin allergies, stomach upsets, especially diarrhoea. Probiotic food concept has just come into limelight with introduction of some of dairy products. However, presently Indian market is at infancy stage. Leading players in India, who are engaged in Probiotic Dairy products are Mother Dairy, Amul, Nestle, Britannia and Yakult-Danone. Presently, Mother Dairy's dahi and lassi are available with brand name B-active in National Capital region which has received a very good consumer response due to its acceptable body and texture and having appropriate acidity level. B-active probiotic dahi is available in different pack sizes to suit the different customer requirements. Amul has also entered in to the probiotic segment with the introduction of ice-cream and dahi. Nestle has launched ActivePlus dahi and Yakult-Danone, a probiotic drink Yakult.

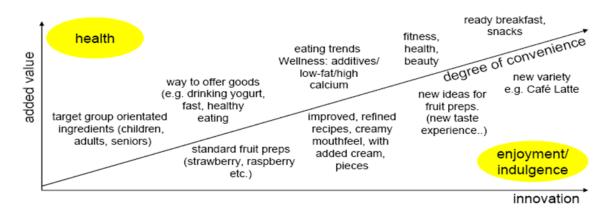
#### **Future Potential**

The growing health-consciousness in newer generation has paved the way for varieties of weight reducing, low-cholesterol, high fiber, nutrient-rich food products in recent times and the demand of such products has increased manifold during recent times. Manufacturers will respond by introducing new products that will add more value to their existing portfolios. India is becoming an attractive destination for investments in this sector. Recent developments in the market, however, indicate a move away from treating serious health problems and diseases to addressing more common, lifestyle issue. In terms of new product launches, there is a strong emphasis on wellness products marketed with "soft" health claims. Challenges for Food industry

Vast opportunity exists for food manufacturers to formulate a whole new generation of probiotic-containing products. Such an endeavor should consider:

- Choice of strain or strain combinations
  - This should be based on evaluations of health effects, mechanisms of action, and stability characteristics of the specific strain using validated biomarkers in *in vitro*, animal, and human subject studies.
- Definition of a physiologically relevant dose consumption levels
  - Dose which research suggests provides a beneficial health effect or reduced risk of disease.
- Consumer communication program
  - That discusses the basis of probiotic functionality and specifics of the probiotic product.
- Package labeling
  - Appropriate labeling of the Probiotic product package is essential to communicate approximate numbers of viable cells at the end of shelf life, and the genus, species and strain used. Any health statements, such as structure/ function statements, must comply with FDA regulations. Concern over how to label products with health messages to attract consumer interest while meeting federal regulations
- Compatibility of probiotic with food format.

Probiotic survival in foods is an important consideration in this regard. Factors involved in probiotic survival are complex involving strain genetics, strain cultivation procedures, preservation method, preservation compounds, food composition, storage temperature, and storage time, among others.



Multi directional application of Probiotics as lifestyle related issues/problems

#### PROBIOTIC EFFICACY DELIVERY FORMATS

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With growing interest of probiotics, many people are eager to use this approach for prevention and treatment of various human diseases. It is important that

recommended levels of viable microorganisms must be consumed daily to maintain therapeutic effect on human **Probiotics** beings. available nutraceuticals, are freeze dried cultures consumed as dietary supplements or packed in capsules. Foods and beverages are increasingly popular carriers of probiotics particularly, dairy products such as cheese, yogurt or dahi, ice cream and other dairy desserts are considered as an ideal vehicle for delivering probiotic

Food constituents play vital role in probiotic growth and survival in food and during transit in gastrointestinal tract. They buffer the bacteria through the stomach

bacteria to the human gastrointestinal tract. Recently, several non-dairy based products such as fruit juices, chocolates, vegetables, legumes and cereal products are

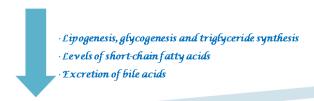
also tried for probiotic delivery. Food constituents play a vital role in probiotic growth and survival in food and during transit in gastrointestinal tract. They buffer the bacteria through the stomach and may contain other bioactive ingredients that could interact with probiotics that might augment or diminish the efficacy. In addition, the interactions determine the specific cell components and cell physiology, thereby, alter the probiotic functionality. In many clinical studies of probiotic functionality, the most documentation available for probiotics is when they are supplied in a dairy matrix, either fermented or unfermented milk. Although, biomarkers or health benefits attributed to yogurt are available, limited reports exists for other food products including non-dairy. Furthermore, few reports are available with little differentiation of the effects of probiotics administration in food matrices and supplement forms. A recent article appeared in a peer reviewed journal summarizes the need for considering the delivery matrix formulations and their effect on probiotic functionality for advancement of probiotics research.

# Research Updates

#### Therapeutic approach for manipulating gut microbiota in the management of obesity and type 2 diabetes

#### Kootte et al., Diabetes Obes Metab. 2011

In this article, the authors have reviewed extensively various biotherapies like probiotics, prebiotics, short chain fatty acid and pharmaco-therapies like antibiotics together with microbial transplantation in the management of metabolic syndromes like obesity and type 2 diabetes. The comparison of metabolism in germ free animal and conventionally raised animal shows that enterotype of host may improve the capacity to harvest energy and also may contribute to obesity and other metabolic syndroms. So gut microbiota is hypothesized to influence total body energy homeostasis. Authors reviewed the direct contribution of gut microbiota in SCFA and bile acid metabolism and their further intervention on host metabolic pathways. This review concluded that, some of these therapies will modify / manipulate gut microbiota driven pathways in favorable proportion that can help in the management of metabolic syndrome.



- · Cholesterol content
- Levels of conjugated bile acids (downregulated deconjugation)
- · Concentration of bile acids in bile
- Insulin sensitivity
- · Reabsorption of (conjugated) bile acids
- Excretion of triglycerides, cholesterol and total lipids

#### Page 7

#### New probiotic strains will win health claims (but not for 10-15 years): Prof Dirk Haller

Advances in genomics and understanding the human gut microbiota will lead to the discovery of probiotic bacterial strains that will deliver biomarker-measured health benefits – but it may take 10-15 years, according to the leading researcher.

Source: http://www.nutraingredients.com. For more information read the complete article using following link.

http://www.nutraingredients.com/On-your-radar/Probiotics/New-probiotic-strains-will-win-healthclaims-but-not-for-10-15-years-Prof

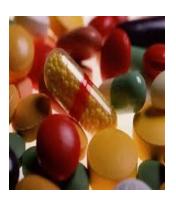


Prof. Dirk Haller

Whole-Genome Shotgun Sequencing of an Indian-Origin Lactobacillus helveticus Strain, MTCC 5463, with Probiotic Potential

#### Prajapati et al., 2011, J Bacteriol., 183: 4282-4283

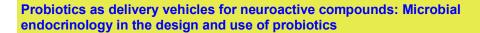
Lactobacillus helveticus MTCC 5463 was isolated from a vaginal swab from a healthy adult female. The strain exhibited potential probiotic properties, with their beneficial role in the gastrointestinal tract and their ability to reduce cholesterol and stimulate immunity. The authors sequenced the whole genome and compared it with the published genome sequence of Lactobacillus helveticus DPC4571.



# The new link between gut-brain and neuropsychiatric disorders

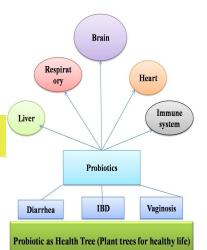
# Fetssov, S. O and Dechelotte, P, Curr Opin Clin Nutr Metab Care. 2011 Sep;14 (5):477-82.

In this review, the authors summarized the findings related to origin of eating disorders and neuropsychiatric disorders in an attempt to establish the new link between gut and brain to study the neuropsychiatric conditions including anxiety, depression, eating and sleep disorders. The authors specifically focused on resemblance between gut microbial proteins and several key neuropeptides that involved in the regulation of motivated behavior and emotion. Finally, the authors concluded that alteration in the link/signal between gut and brain may contribute to several neuropsychiatric disorders.



#### Mark Lyte. 2011, Bioessays 33: 574-581.

In this hypothetical review, the author hypothesized the ability of probiotics to synthesize neuroactive compounds that can affect the host's gastrointestinal and psychological health through receptor-based targets on immune and neuronal elements (intestinal and extra-intestinal). This unifying microbial endocrinology-based hypothesis may facilitate the selection and design of probiotics for clinical use. This also highlights the largely unrecognized role of neuroscience in understanding how microbes may influence health.





### Chr-Hansen- A Profile

CHR Hansen is a global supplier of bioscience based ingredients to the food, health and animal feed industries, with leading position in the markets in which we operate. We produce cultures, dairy enzymes, probiotics and natural colors.

Our market positions are built on our product innovation and applications, production processes, long-term customer relationships and intellectual property.

Key focus areas include the development, production and sale of cultures, enzymes and probiotics for the food industry.

- DVS Cultures for Set Yoghurt( DAHI), Fruit Yoghurt, Stirred Yoghurt, Lassi, Buttermilk and other dairy desserts
- Probiotic Cultures- a range of proprietary documented strains with documented health benefits like improved digestive system,
   balanced intestinal flora and improved immune system
- DVS Cultures & Dairy Enzymes for Cheese applications.

Chr- Hansen supplies Antibiotic Test Kits to check for antibiotic residues in milk.

Key focus areas include the development, production and sale of products with dietary supplement, over the counter pharmaceutical, infant formula and animal feed industries.

Other focus areas includes the provision of natural colours solutions to the food industry focusing on beverages, confectionery, ice cream, dairy products and fruit preparations.

#### **Meeting Consumer Demand**

We engage in more than 1,000 development projects with customers each year at Chr. Hansen's applications and development centres around the globe.

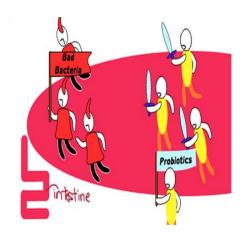
Our Customers get:

- Quick access to specialist consulting
- In-depth understanding of market trends
- Innovative ingredients backed by extensive R&D studies
- Individually adjusted solutions
- High quality standards
- Quick access to 19 application and development centres worldwide

#### Customer - driven innovation

As a knowledge- based company, our research and development activities occupy a crucial and exciting role in the company. It is our strategy to continually develop revolutionary new products and concepts that enable our customers to follow their visions and launch new innovative products.

www.chr-hansen.com



# HATS OFF PROBIOTICS

Man is the dazzling creature
With lots of amazing features
Blessed by hage power of brain
As Gods blessing shower or rain

if we want
healthy nation
then everybody
must have
probiotic in his
ration

Once people's brain miserably frozen

Caught by villain Pathogen

But misfortune of scoundrel

Brain graced by probiotics mission

Its called an army against many disease

Due to it Diarrohea, Cancer, n Diabetes too seizes

Its found to helpful in H. Pylori treatment too

Avoiding side effects of such, letting human to be glow

Therefore Probiotics are desperately essential

Role of Probiotic is very crucial

HATS OFF PROBIOTICS HATS OFF PROBIOTICS

**Snehal Katkamwar** 



### The PAI family (Member details) after the release of 1st issue

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