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POTENTIAL OF PROBIOTICS AND PREBIOTICS FOR SYNBIOTIC FUNCTIONAL DAIRY FOODS: AN OVERVIEW

Ravinder Nagpal, Hariom Yadav, Anil Kumar Puniya, Kishan Singh, Shalini Jain and Francesco Marotta

ABSTRACT: Conventionally, food healthiness has been associated with nutritional factors such as fat, fiber, salts and vitamins. In addition to this traditional healthiness, food may contain single components that may have a positive impact on our well-being. Food processing and biotechnology has enabled the food industry to make food with special characteristics. Probiotics, prebiotics, synbiotics and functional foods have been created to describe food products with special characteristics. Functional Foods are generally described as foods that promote health beyond providing basic nutrition. These foods are eye-catching to modern men and women who wish to reach old age in a healthy shape. The best known examples of functional food are fermented milks containing probiotic bacteria. Such functional foods provide a novel approach to the idea of healthy eating by linking a single component with a certain health effect in a single product. In the last few years, great attention has been dedicated to probiotics and prebiotics or their combined use (synbiotics) in improvement of human health in a natural way because of their history of safe use and the general body of evidence that supports their positive roles. Such synbiotics indicate a realistic way of using biological preparations in the prevention of gastrointestinal diseases in humans.

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METHODOLOGICAL ASPECTS IN PROBIOTIC RESEARCH – A BRIEF REVIEW WITH AN EMPHASIS ON EPITHELIAL BARRIER FUNCTION

Michael Schultz and A. Grant Butt

ABSTRACT: Emerging data from recent studies clearly demonstrate that there is an array of tools available to study probiotic effects. However, the tools have to be chosen carefully to reach appropriate conclusions. Cell lines such as Caco-2, HT-29 or T84 are remote from the human system and due to their origin from immortal cancer cell lines, are artificial. However, they are readily available and not only allow first impressions but also more in depth analysis of pathways of underlying mechanisms of known or newly discovered probiotic strains. Extrapolation into the human system has to be done with care. Animal models, although more closely related to the human system, need to be chosen wisely to allow investigation of the desired effect. Most animal models of experimental colitis such as different knockout models are highly susceptible to environmental changes, especially the microbiological flora and have to be established at the site of investigation first. Whether the resulting immunomodulation following probiotic therapy is a primary effect or just subsequent to amelioration of colitis has to be determined individually. Due to the complexity of the human system with numerous uncontrollable variables, sophisticated investigations into immunomodulation upon probiotic therapy is difficult but has been attempted. Special situations such as pouch formations seem to be favourable. The probiotic market is confusing with reports of beneficial effects, exerted by probiotics coming out in abundance. It will be exciting to watch which probiotics will emerge as clinically relevant in the future.

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UTILIZATION OF ARABINOGALACTAN, ALOE VERA GEL POLYSACCHARIDES, AND A MIXED SACCHARIDE DIETARY SUPPLEMENT BY HUMAN COLONIC BACTERIA *IN VITRO*

Robert A. Sinnott, Jane Ramberg, Jakob M. Kirchner, Cherie Oubre, Christy Duncan, Stephen Boyd, and John E. Kalns

ABSTRACT: We hypothesized that a small subset of colonic flora would be able to utilize complex plant polysaccharides (CPPs). Feces from 4 healthy humans were sequentially passed three times over 9 days in growth media composed of either aloe vera gel polysaccharides (AVP), larch arabinogalactan (LAG), or a dietary supplement including these two ingredients (MSS) suspended in a minimal salt solution. CPPs were first dialyzed to eliminate polymers and sugars <8,000 MW, forcing bacteria to consume only higher MW components. Isolates were obtained on rich media and then 16S DNA sequenced for species identification. API strips were in some cases used to confirm identification. A total of 6 species were identified; however, 90% of isolates were Enterococci. Supernatant analysis showed variable consumption of the entire MW range of polysaccharide components. These findings suggest that enterococcus species play an important role in the utilization of CPPs.

PROBIOTIC PROPERTIES OF *LACTOBACILLUS PLANTARUM* CH1 ISOLATED FROM INDIAN GREEN SAUCES

Simarata Dhillon, Moushumi Ghosh and Abhijit Ganguli

ABSTRACT: We evaluated the probiotic characteristics of lactobacillus strains isolated from popular, non-dairy Indian foods viz; pickles and green sauce. One strain of lactobacillus selected on the basis of high tolerance to bile was characterized by conventional and molecular methods as *Lactobacillus plantarum*. This strain was further tested for (in vitro) pattern of antibiotic resistance, tolerance to artificial gastric juice, enzymatic profile, cholesterol reduction and antagonistic activity against enteric pathogens. The strain were susceptible to 4 out of 11 antibiotics tested, survived at high bile salt concentration (2% oxgall) and artificial gastric juice. The strain reduced cholesterol by 80% and completely inhibited all the enteric pathogens tested. The results of this study suggest *Lactobacillus plantarum* Ch1 to be a potential probiotics strain.

PROTECTIVE ROLE OF TWO LACTOBACILLUS BACTERIA SPECIES AGAINST THE GENOTOXICITY INDUCED BY THE MYCOTOXIN DEOXYNIVALENOL

MS Hassanane, KB Abdel Aziz, EAM Abd Alla, M Zaher, I Yehia, and HM Ali

ABSTRACT: Two probiotic bacteria named: *Lactobacillus gasseri* and *Lactobacillus reuteri* were tested for their ability to reduce the possible genotoxic effect induced by the mycotoxin deoxynivalenol (DON). The evaluation conducted on 78 adult male laboratory mice at mitotic and meiotic chromosomes as well as sperm count and morphology. The evaluation performed after treating the animals with three doses of toxin equal to the (1/10, 1/8, 1/4) fractions of the LD50. The two probiotic bacteria were also tested alone. The results showed that DON causes a significant reduction in mitotic and meiotic activity. Additionally it causes structural and numerical abnormalities in both bone marrow and spermatocytes, it also induced sperm shape abnormalities. Adding the two types of the probiotics separately improve the situation and reduced the incidence of somatic, germ cell chromosome abnormalities, sperm abnormalities were also reduced. The protective role of the probiotic *Lactobacillus gasseri* is proved to be slightly more than the other probiotic *Lactobacillus reuteri*. The two types of the probiotics didn't show any significant mutagenic activity when they tested alone confirming adding them to the safe list of probiotics.

IN VITRO EVALUATION OF LACTIC ACID BACTERIA AS POTENT PROBIOTICS FOR NUTRACEUTICAL APPLICATIONS

K.B. Praveen Kumar Reddy and S.G. Prapulla

ABSTRACT: Over the past 5 years, the probiotic field has exploded with a number of new cultures, each purported to elicit a variety of benefits. Scientifically established health claims remain among the highest priorities to those who seek to establish solid health benefits that will promote a particular probiotic brand. The scientific community faces a greater challenge and must objectively seek, cause and effect relationships for many potential and currently investigated probiotic species and strain combinations. In this context 13 lactic acid bacteria were evaluated for their applicability as putative probiotics in fermented dairy products. Out of the 13 LAB cultures, *L. plantarum* B-4496 and *L. salivarius* CFR-2158 showed more than 70% survivability at pH 2, 82% and 90% survivability at pH 2.5 and 80% survivability at 0.3% bile salt. Among 13 LAB, *L. casei* NCIM-2586, *L. helveticus* B-4526, *L. acidophilus* B-4495 and *L. salivarius* CFR-2158 significantly reduced the cholesterol to 90.50%, 85.59%, 84.6% and 81.33% in the presence of 0.3% of bile salt respectively. Also, LAB cultures showed β -galactosidase activity and good antibacterial activity against Enterotoxigenic *E. coli* (ETEC) that is the main culprit in causing traveler's diarrhea. Considering all the probiotic properties, *L. plantarum* B-4496 and *L. salivarius* CFR-2158 appear as promising candidates to be considered as a probiotic supplement in food products.

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IMMUNOMODULATORY POTENTIAL BY ORAL FEEDING OF PROBIOTIC DAHI CONTAINING *LACTOBACILLUS ACIDOPHILUS* AND *LACTOBACILLUS CASEI* IN MICE

Shalini Jain, Hariom Yadav and PR Sinha

ABSTRACT: In the present investigation, the effect of probiotic dahi containing *Lactococcus lactis* ssp biovar diacetylactis 60 and two probiotic bacterial strains *Lactobacillus casei* 19 and *Lactobacillus acidophilus* 14 on the non-specific immune response in mice was evaluated. Oral administration of dahi showed 85% increases in β -galactosidase activity of peritoneal macrophages as well as in cultured peritoneal macrophages (117%) after 2 days feeding as compared with unfermented milk fed animals. No significant differences were observed in β -glucuronidase activity in the supernatant of cultured macrophages between unfermented milk and dahi fed animals. However, after 2 days of experimental period a slight increase was observed in the β -glucuronidase activity of peritoneal fluid collected from animals fed with dahi, thereafter values come near to control. The phagocytic activity was also higher in mice fed with dahi during 8 days as compared to skim

milk fed group. There was also increase in lactobacilli counts in intestine of mice fed with dahi as compared to skim milk. It showed that dahi containing probiotic bacteria given orally to mice are able to activate macrophages greater than skim milk and it is suggested that these bacteria when passed through the intestinal tract may be responsible for enhanced gut immune system.

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EVALUATION OF A PROBIOTIC DAIRY PRODUCT FOR ANTIULCER ACTIVITY IN RATS

Majji Neelima, Doddola Sujatha, Koganti Bharathi, Anil PA Kumar and Koganti V.S.R.G. Prasad

ABSTRACT: A marketed probiotic dairy product (curd) containing *Bifidobacterium lactis*, *Lactobacillus acidophilus* and *Lactobacillus casei* each at a count of 10^6 - 10^8 cfu/g was evaluated for antiulcer activity in different models of gastric ulceration in rats. Gastric ulcers were induced by pylorus ligation method, stress-induced, ibuprofen-induced and ethanol-induced models. Probiotic dairy product was administered in the dose of 5 g/ animal for 30 days prior to ulcer induction. Mean ulcer number, size and index were determined. Volume of gastric juice, total and free acidity were estimated in the pylorus ligated rats. Ranitidine was used as a reference drug. The probiotic dairy product showed significant antiulcer activity in pylorus ligation model. However, the antiulcer activity was less than that of ranitidine.

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ENTRAPMENT OF PROBIOTIC BACTERIA WITHIN ALGINATE-HPMC FLOATING BEADS, THEIR SURVIVAL AND EFFECTIVENESS AGAINST ETHANOL INDUCED ULCERS IN RATS

I.P. Kaur, A. Garg, T. Geetha and R. Agrawal

ABSTRACT: Probiotics are nonpathogenic microbe used to confer health benefits on the recipient. The derangement of normal body flora has been held responsible for causation of various disorders. Oral administration of probiotics has been proved beneficial in various gastrointestinal disorders such as ulcers, diarrhea and colonic cancers. To act as probiotics, the bacteria must arrive in the intestine alive and in sufficient number. The significance of survival of probiotics in the GI-tract, their translocation and colonization and the fate of probiotic-derived active components indicate a need and scope of packaging them into a suitable delivery system. Further, for an effective ulcer healing, a local therapeutic action with a long duration is desirable. Floating drug delivery system forms the most promising delivery system for antiulcer agents. The purpose of the present study was to incorporate probiotic (*L. acidophilus*) into floating beads (sodium alginate: HPMC :: 9:1 w/w) and establish the viability of the entrapped probiotic. The former would ensure a prolonged and continuous release of the probiotic in the stomach allowing sufficient time for its adhesion onto the gastric mucosa, which is a major limitation to probiotic therapy.

Calcium carbonate was used as a gas forming agent (25%-75%). In-vivo evaluation in ethanol induced ulcers (in male Wistar rats) in terms of ulcer index (ulcer index was reduced from 6.00 to 2.00) and oxidant status of stomach homogenates indicated a significant effect. The study indicates the suitability of using probiotics especially when formulated as floating beads for effective healing of gastric ulcers.

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BIFIDOBACTERIA PREVENTS NECROTIZING ENTEROCOLITIS AND INFECTION IN PRETERM INFANTS

Yayoi Satoh, Koichi Shinohara, Hikaru Umezaki, Hiromichi Shoji, Hiroaki Satoh, Yoshikazu Ohtsuka, Seigo Shiga, Satoru Nagata, Toshiaki Shimizu, and Yuichiro Yamashiro

ABSTRACT: Necrotizing enterocolitis (NEC) is one of the leading causes of morbidity and mortality in extremely and very low birth weight (ELBW and VLBW) infants. These premature infants are more susceptible to infections and to death from infectious diseases than term infants. To investigate protective effects of *Bifidobacterium breve* (B. breve) as probiotics from NEC and infection in these premature infants, we carried out a clinical study. Three hundreds and thirty eight ELBW and VLBW infants (220 ELBW and 118 VLBW), admitted at our NICU during a five year period between January 1999 and December 2003, were enrolled in this study. These patients were supplemented with B. breve initiating from several hours after birth (Bifido group). Two hundreds and twenty six premature infants (101 ELBW and 125 VLBW), who were not given B. breve, admitted at our NICU during a five year period from January 1994 to December 1998 were served as a historical control. Infants of the Bifido group were administered daily B. breve, a freeze-dried preparation dissolved in raw own mother's milk or formula for premature infants, with a dose of 1×10^9 cells/day. Administration of B. breve was divided into two doses through naso-gastric tube. The incidence of NEC was significantly reduced in the Bifido group (nil) compared with that in the historical control group (6 cases, $p < 0.01$). Regarding infection, not only a significant reduction of the morbidity rate (28.8% in the control to 20.7% in Bifido group, $p < 0.05$) but also the mortality rate (13.8% to 0.6%, $p < 0.01$) and the mortality by infection in the total mortality were observed. The beneficial effects of probiotics in premature infants were also reconfirmed in our latest observation. Administration of B. breve as probiotics is a very effective treatment to prevent from NEC and infection in ELBW and VLBW infants.0000

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VAGINAL CAPSULES OF *LACTOBACILLUS PLANTARUM* P17630 FOR PREVENTION OF RELAPSE OF CANDIDA VULVOVAGINITIS: AN ITALIAN MULTICENTRE OBSERVATIONAL STUDY.

C. Carriero, V. Lezzi, T. Mancini, L. Selvaggi and the Italian multicentre study group

ABSTRACT: To evaluate the efficacy of *Lactobacillus plantarum* P17630 as vaginal probiotic in preventing recurrence of *Candida* vulvovaginitis, an observational, multicentre, prospective study on women aged 18-45, with symptomatic non-complicated vulvovaginal candidiasis (VVC), has been conducted: 476 patients were available for analysis: 252 in the study group, i.e. oral fluconazole 150 mg “one-shot” treatment + soft-gel vaginal capsules containing *Lactobacillus plantarum* P17630, following the menstrual periods; 224 in the control group (fluconazole treatment alone). Clinical data were compared at baseline (T0), after 4 weeks (T4W), and after 4 months (T4M). Statistical analysis was performed using Student’s t test, Wilcoxon’s test and chisquare test. At T4W the proportion of asymptomatic patients was significantly higher in the lactobacilli group than in the control group (82.9%% vs. 71.9%, $p=0.003$). At T4M follow-up VVC relapse was registered only in 22 cases out of 252 in case group, while 59 out of 224 in control group showed recurrence: OR= 3.74 (2.20-6.34 95% C.I.) and $p<0.001$. This study suggests that the addition of vaginal *Lactobacillus plantarum* P17630 to oral fluconazole may enhance therapeutic efficacy in terms of both treatment and prevention of episodes of vulvovaginal candidiasis during a short-term follow-up.

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EFFECTS OF LACTITOL COMPARED TO POLYETHYLENE GLYCOL-3350 ON FECAL SHORT CHAIN FATTY ACIDS AND MICROFLORA IN OLD PATIENTS WITH IDIOPATHIC CHRONIC CONSTIPATION

Daniele Scevola, Piero Marone, Vincenza Monzillo, Filippo Uberti, Silvia Scevola and Giuseppe Cetta

ABSTRACT: Chronic idiopathic constipation and consequent abuse of laxatives are common in the elderly. The disaccharide lactitol and polyethylene glycol-3350(PEG) are among the most frequently prescribed agents in the treatment of constipation because of their good safety profile and efficacy. No comparative studies have been conducted on the effects of the two drugs upon fecal content of bacteria, short chain fatty acids (SCFAs) and enzymes in old people with constipation. To demonstrate that lactitol, compared to PEG, improves chronic idiopathic constipation in the elderly patients by altering fecal bacterial counts, SCFAs and enzymes content of the stools, thirty subjects (10 males and 20 females), aging >60 years (mean= 66.4 years \pm 5.5), with chronic idiopathic constipation (< 3 spontaneous bowel movements per week) were enrolled to receive 20g of lactitol (Portolac® or 15g of PEG-3350 (Movicol®) a day as a laxative for four weeks after a washout week without laxatives, prebiotics or probiotics. The study was an open, randomized, parallel groups, third party (laboratory) blinded trial. The effects of both substances on fecal SCFAs, bacterial

microflora, water, pH, proteins, starch, fat, sugar, α -galactosidase and β -glucuronidase activity, number and consistency of stools, intestinal symptoms, were evaluated. Compliance and clinical efficacy were similar and all subjects with both treatments experienced an increase in the number and a decrease in the consistency of stools per day. Total fecal SCFAs, and particularly acetate and butyrate concentrations increased with lactitol and fell with PEG ($p < 0.05$). In lactitol group Bifidobacteria and Lactobacilli counts correlate with total SCFAs ($r = 0.48, p < 0.05$), acetate ($r = 0.50, p < 0.05$), propionate ($r = 0.44, p < 0.05$), and respectively with total SCFAs ($r = 0.44, p < 0.05$), butyrate ($r = 0.46, p < 0.05$), isobutyrate ($r = 0.69, p < 0.001$), valerate ($r = 0.53, p < 0.01$) and isovalerate ($r = 0.58, p < 0.005$). In PEG group the correlations resulted significant between Enterobacteria and total SCFAs, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate. The α -galactosidase was significantly increased by lactitol and decreased by PEG ($p < 0.05$). β -Glucuronidase fell with both treatments ($p < 0.05$). pH from baseline value of 7.5 ± 0.7 turned toward acidity (6.6 ± 0.8) with lactitol and from 7.4 ± 0.7 toward alkalinity (7.7 ± 0.5) with PEG. Lactitol and PEG both normalize the frequency of evacuations but with different mechanisms. Lactitol at nonosmotic dosage works as a prebiotic increasing stool SCFA concentrations, particularly butyrate and acetate, while PEG, as pure osmotic laxative, negatively interferes with colonic fermentation reducing SCFA production.