

# INTERNATIONAL JOURNAL OF PROBIOTICS & PREBIOTICS

VOLUME 6

NUMBER 2

May 2011

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Ehab Essa Kheadr, Nassra Dabour, Geneviève Petit and Jean-Christophe Vuillemand

**ABSTRACT:** *This study was undertaken to evaluate the ability of probiotics in a Saint-Paulin type cheese and in a fermented milk drink to survive during their transit through a dynamic model of the human digestive system as well as the effectiveness of those commercial products to deliver living probiotic cells to the colon. The Saint-Paulin cheese contained Lactobacillus paracasei as adjunct probiotic culture, while fermented milk contained both Lactobacillus rhamnosus and Bifidobacterium longum. Fresh and 30-day-old cheese and 45-day-old fermented milk samples were digested in a dynamic gastrointestinal model. Although mortality rates of the probiotic strains were important during the passage through the gastrointestinal tract (>98%), results showed that ingestion of 30 g serving of cheese or 175 g of the fermented milk could deliver more than 10<sup>7</sup> viable cells of probiotic bacteria to the colon.*

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Jitender Mehla and SK Sood

**ABSTRACT:** *Pediococcus pentosaceus NCDC 273, a dairy strain was screened for production of an YGNGV motif containing antimicrobial peptide (AMP). In microbiological and PCR based screening, it was shown that P. pentosaceus NCDC273 produces an AMP i.e Pediocin 273, encoded by pedA gene. The molecular weight of pediocin was determined about 5.19 kDa. The purification yield was 438% and the specific activity was increased 413 folds. Till now this is the highest purification yield obtained for any purified pediocin. During purification, it was shown that the peptide was having enhanced antimicrobial activity at acidic pH than at neutral pH. Both examination of the inhibitory spectrum and the characterization of AMPs do not give sufficient and exact information to distinguish between different pediocins. Therefore, it may be recommended to include PCR based screening as a preliminary step to detect new pediocins or more accurately AMPs. In Silico analysis showed that pediocin 273 is a positively charged, hydrophobic, YGNGV motif containing antimicrobial peptide. Pediocin 273 suggests a great potential in applications in food industry, pharmaceuticals and biomedicine.*

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### 89-94 EFFECT OF SUPPLEMENTATION OF PROBIOTIC *BACILLUS COAGULANS* UNIQUE IS-2 ON HYPERCHOLESTEROLEMIA SUBJECTS: A CLINICAL STUDY

M. Ratna Sudha, Neelam Radkar and Asin Maurya

**ABSTRACT:** *The objective of this study was to evaluate the effect due to consumption of capsule containing Bacillus coagulans Unique IS-2, on serum lipids for 60 days. Thirty hyperlipidemic (Having serum cholesterol levels of more than 200mg/dl) subjects were divided into 3 groups (n=10). Two group subjects were allotted to receive a daily dose of two capsules of probiotic Bacillus coagulans Unique IS2 (10X10<sup>9</sup>CFU/capsule (Group A) and 20X10<sup>9</sup>CFU/capsule (Group B)) and third group subjects were received standard medication. Serum lipid profile was done at 0, 30 and 60<sup>th</sup> day of the study period. At the end of study there were slightly reductions in total cholesterol (11%) and LDL (0.8%), whereas an increase in HDL cholesterol levels (3.6%). This data suggest that the strain Bacillus coagulans IS-2 has the potential of control in the serum cholesterol in humans suffering with hyperlipidemia.*

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### 95-100 ISOLATION AND IDENTIFICATION OF *ENTEROCOCCUS* SP. FROM STOMACH OF HONEYBEE BASED ON BIOCHEMICAL AND 16S rRNA SEQUENCING ANALYSIS

N. Tajabadi, M. Mardan, M. Shuhaimi and M.Y. Abdul Manap

**ABSTRACT:** *This is the first report of isolation and identification of five strains of Enterococcus from honey stomach of the Giant honeybee Apis dorsata. Samples of honeybee were collected from Apis dorsata colonies in different bee trees and Enterococcus isolated from honey stomach using selective media. The forty-three isolates were Gram-stained and tested for Catalase reaction. The 16S rRNA genes from extracted DNA of bacterial colonies were amplified with polymerase chain reaction (PCR) using universal primers. All bacterial 16S rRNA genes were sequenced and the distant bacteria deposited in GenBank. The Enterococcus strains were identified as Enterococcus sp. named Taj-KS29, Taj-KH5, Taj-TS315, Taj-TS329 and Taj-TS355. However, it appears that honey stomach of honeybee could be a source of new lactic acid bacteria.*

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### 101-108 EVALUATION OF THE *IN VITRO* AND *IN VIVO* PROBIOTIC QUALITIES OF LACTIC ACID BACTERIA (LAB) RECOVERED FROM LOCALLY FERMENTED PRODUCTS

Anteneh Tesfaye, Tetemke Mehari, and Mogessie Ashenafi

**ABSTRACT:** *From 143 lactic acid bacteria (LAB) recovered from locally fermented products, 27 strains were antagonistic against sensitive LAB indicator (Lab. brevis 3). Based on in vitro (acid-bile tolerance and antagonism against foodborne pathogens) criteria 9 LAB were selected for in vivo mice model infection test. LAB (pure or mixed cultures) and Salmonella Typhimurium DT104 were administered orally to mice at log 6 and log 4 cfu/ml, respectively. The count of Salmonella Typhimurium DT104 from the feces of co-infected mice was*

significantly reduced ( $p < 0.01$ ) by mixed LAB culture (MLC) 3, MLC 2, MLC 1, *Lab. plantarum* 1, *Ped. pentosaceus* 1, *Lab. delbreuckii* ssp *delbreuckii*, *Lab. brevis* 1, *Lab. acidophilus* 1 and *Lab. plantarum* 2. MLC 3 and 2 totally eliminated the test pathogen from the feces co-infected mice at day 15 and 16, respectively. The effects of MLCs were found better than single LAB cultures. Besides, the effect of single LAB cultures was improved much at log 9.00 cfu/ml administrations. Both the *in vitro* and *in vivo* probiotic analyses results strongly suggest that LAB, most single and all MLCs are possible candidate probiotics that can be used for the formulation of starters to prepare safe and bioprotective products.

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109-114 *IN VITRO* ADHESION AND *IN VIVO* VITAMIN B<sub>12</sub> STUDIES WITH POTENTIAL PROBIOTIC STRAIN *PROPIONIBACTERIUM FREUDENRECHII* SUBSPS *SHERMANII* (OLP-5)  
Thirupathaiah Yeruva, Swarupa Rani Chiliveri, Sudhakara Reddy  
Marrivada, Smita Hasini Panda and Venkateswar Rao Linga

**ABSTRACT:** *Propionibacterium freudenreichii* subsps. *shermanii* (OLP-5) along with three standard strains (NRRL-B-3524, 3523 and 4327) were used to study the *in vitro* adhesion properties for human intestinal epithelial cell lines (HT-29) and porcine intestinal mucus. The OLP-5 strain was also used to determine *in vivo* vitamin B<sub>12</sub> levels in experimental animals. *Propionibacterium* strains exhibited a level of adhesion between 12 to 25 % to HT-29 cell lines. Adhesion to mucus was observed higher (28-38%) than epithelial cell line HT-29 and it is likely that the adhesion to mucus is the result of non-specific interactions. At the end of animal studies the serum vitamin B<sub>12</sub> level of the rats fed with OLP-5 was significantly higher (655 pg/ml) than that of the Deficiency group (280 pg/ml). Our strain (OLP-5), being tolerant to low pH and moderate concentration of bile salts, this adhesion property and enhanced vitamin B<sub>12</sub> in animal experiments adds to its possible probiotic applications. Above observations, also indicate that HT-29 cell lines can also provide an alternative to Caco-2 cell lines for assessing *in vitro* adhesion properties of probiotic *propionibacterium* strains.

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115-122 PROBIOTIC FERMENTED SOY PRODUCT STIMULATED CYTOKINES IN RATS ON A LONG-TERM CARCINOGENIC MODEL  
Fernanda Paulin Benzatti, Katia Sivieri, Elizeu Antonio Rossi and Iracilda Zeppone Carlos

**ABSTRACT:** *Lactic acid bacteria* (LAB) when used as probiotics can promote stimulation of immune system through increase of the pathogen phagocytosis and also the cytokines production. The purpose of the present work was to evaluate cytokines production by peritoneal macrophages and splenic lymphocytes in rats with colon cancer induced 1,2 dimethylhydrazine (DMH) after treatment with soy product fermented with *Enterococcus faecium* CRL 183 supplemented with or without calcium. A total of 30 male Wistar SPF rats, randomly allocated to 6 groups (n=10), received the following treatments: Group 1- control; Group 2 -Positive control, rats with colon cancer induced with DMH; Group 3 -DMH rats treated with fermented soy product; Group 4- DMH rats treated with fermented soy and calcium; Group 5- rats treated with fermented soy alone; 6 Group- rats treated with fermented soy and calcium. The groups of induced animals received, subcutaneously, 20 mg/kg body

weight of DMH in a weekly dose for 14 weeks. The non-induced animals were inoculated, subcutaneously, with 1mM EDTA (pH 6.5). At the end of the forty-second week the animals were euthanized in a CO<sub>2</sub> chamber. Thioglycollate-elicited peritoneal exudates cells (PEC) were harvested from Wistar SPF rat using 5.0 mL of sterile phosphate-buffered saline (PBS), pH 7.4. The cells were washed twice by centrifugation at 200 g for 5 minutes at 4 °C and resuspended in appropriate medium for each test (H<sub>2</sub>O<sub>2</sub>, NO, TNF- $\alpha$ , IL-6, IFN- $\gamma$  and IL-4). Results showed that in colon cancer animals, which were ingested fermented soy, product there is a higher immune system stimulation observed in increased of the H<sub>2</sub>O<sub>2</sub>, TNF-  $\alpha$ , IFN-  $\gamma$  and IL4 production. Results suggest that ingestion of fermented soy product supplemented or not with calcium favor anti-tumoral response stimulating the cytokines produced by peritoneal macrophages and splenic lymphocytes.

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123-132      **EFFECTS OF THE CONTINUOUS INTAKE OF *LACTOBACILLUS CASEI* STRAIN SHIROTA-FERMENTED MILK ON RISK MANAGEMENT OF LONG-TERM INPATIENTS AT HEALTH SERVICE FACILITIES FOR THE ELDERLY**

Lei Bian, Satoru Nagata, Takashi Asahara, Mohammed Shafiqur Rahman, Toshihisa Ohta, Norikatsu Yuki, Chongxin Wang, Kikuo Takano, Masashi Daibo, Koji Nomoto and Yuichiro Yamashiro

**ABSTRACT:** *We studied the effectiveness of the continuous intake of Lactobacillus casei strain Shirota (LcS)-fermented milk on inpatients living at facilities for the elderly (n=42, 82 ±10 years) in open trials that compared the pre- and post-intake. LcS-fermented milk was taken continuously for 6 months. Feces were sampled and analyses of fecal microflora, organic acid and pH measurement were performed. A reduction in the number of days that the inpatients had a fever, constipation and diarrhea was observed in the post-intake of the milk compared to the pre-intake. In the feces of the inpatients before the intake compared to those of staff (n=24, 40±12 years), Bifidobacterium decreased whereas Clostridium species increased. However, Bifidobacterium proliferated without the detection of methicillin-resistant Staphylococcus in the samples after the intake. No bacteria causing nosocomial infections were detected among the staff. The acetic acid concentration increased and pH decreased in the feces of the inpatients after such intake. LcS-fermented milk is therefore considered to be useful for improving the clinical conditions, and the effects of the enteral microflora and environment in such inpatients. LcS-fermented milk may therefore have efficacy for reducing the risk of infection among elderly individuals residing at nursing homes.*