Probiotics and Antimicrobial Proteins
https://doi.org/10.1007/s12602-019-09529-6

Safety Assessment of Potential Probiotic *Lactobacillus fermentum* MTCC-5898 in Murine Model after Repetitive Dose for 28 Days (Sub-Acute Exposure)

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Abstract
Safety assessment of probiotic *Lactobacillus fermentum* MTCC-5898 (LF) with three doses (10^7, 10^9, and 10^{11} cfu/day/animal) was carried on Swiss albino mouse weanlings for 28 days using oral route. Health status of animals was monitored by physical assessment of body weight, organ indices, and histological appearances of liver and intestine along with measurement of hematological parameters (Hb, WBC, RBC count, MCHC, MCV, MCH), biochemical analytes in blood involving glucose, serum enzymes (ALT, AST and LDH), urea, creatinine, and lipid profile (total cholesterol, triglycerides, HDL, VLDL, LDL, and atherogenic index). LF showed no adverse effects on above parameters of general health status after continuous consumption for the experimental period. On the other hand, significant increase ($p \leq 0.05$) in TGF-β (regulatory cytokine) and considerable decrease ($p \leq 0.05$) in IFN-γ (pro-inflammatory cytokine) without any major changes in IL-4 and IL-12 in intestinal fluid on consumption of 10^9 cfu/animal/day confirmed its dose-specific response for immune homeostasis. Further, safety of LF was also confirmed by insignificant changes in release of FITC-dextran (4 kDa) in blood on its consumption than control group where only saline was given orally. Moreover, significantly ($p \leq 0.05$) increased mRNA expression of *claudin-1* and *MUC-2* in intestinal epithelial cells on feeding *L. fermentum* further supported FITC-dextran permeability data which otherwise showed increased flux of FITC-dextran in blood on consumption of *E. coli* (10^9 cfu/animal/day) due to intestinal damage. Thus, in vivo results confirmed that *Lactobacillus fermentum* MTCC 5898 is safe and non-toxic to weanling mice and may be considered for functional food application after clinical testing.

Keywords Atherogenic index · FITC-dextran · Cytokines · Tight junctions · Gut permeability · *Lactobacillus fermentum*

Introduction

Humans are thought to have evolved a symbiotic relationship with the microbiota of the gut that aid in digestion, contribute to maturation of immune system, counterattack contagious microorganisms, and produce many nutrients such as vitamins (like B_{12}) and short-chain fatty acids [1, 2] thus conferring health benefits. Recently, probiotics have been used to prevent, mitigate, or treat specific diseases. A multitude of clinical trials have examined the use of probiotics for diseases ranging from necrotizing colitis in premature infants to hypertension in adults [3, 4]. The safety of probiotics is tied to their intended use as they are alive when administered and unlike other food or drug ingredients possess the potential for infectivity or in situ toxin production. Safety outcomes of probiotics are inconsistently reported in clinical trials, and their associated adverse events have been recently reviewed by Doron and Snydmanseen [5]. Probiotic safety includes consideration of potential vulnerability of the consumer or patient, dose and duration of consumption, and both the manner and frequency of administration. The US Food and Drug Administration (FDA) has granted GRAS (generally recognized as safe) status to certain probiotic organisms when added to food [6]. However, some theoretical adverse risks have been raised with respect to the use of probiotics in humans which include potential for transmigration causing bacteremia and sepsis. Similarly, colonization with probiotics may have a negative impact on gastrointestinal physiology, metabolism, or immunological functions including potential for antibiotic resistance [7]. To address this predicament, World Health Organization has developed guidelines for the evaluation of probiotics in food, which

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Published online: 08 March 2019