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Adherence capability and safety assessment of an indigenous probiotic strain *Lactobacillus rhamnosus* MTCC-5897

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ABSTRACT

With the growing interest in probiotic microorganisms based on their well established immense health benefits, the present investigation was aimed to assess the adhesion potential and safety of probiotic *Lactobacillus rhamnosus* MTCC- 5897 (LR) before it can be put into a probiotic formulations. *L. rhamnosus* showed an adhesion index of 166.7 ± 11, which was further confirmed by scanning electron microscopy and relative expression of mucus binding protein (*Mub*) and mucus adhesion promoting protein (*Map-A*) genes. *In vitro* safety assessment by tetrazolium dye reduction, neutral red and lactate dehydrogenase (LDH) release assays revealed unchanged metabolic activity of Caco-2 cells even when incubated with *L. rhamnosus* ranged between 10^6-10^{10} cfu/mL for 24 h. Similarly, a moderate increase in bile salt hydrolase (*bsh*) expression (6.84 ± 0.73 and 3.42 ± 0.39 folds in 1% and 3% bile medium respectively) further proved its safety towards normal lipid digestion and absorption. Moreover, *L. rhamnosus* feeding to mice (10^7, 10^9, 10^{11} and 10^{13} cfu/animal/d) repetitively for 28 days revealed no adverse effects on parameters of general animal health status including body weight, organ indices, plasma glucose, liver malondialdehyde (MDA), serum aspartate amino transaminase (AST), cholesterol, triglycerides, high-density lipoprotein (HDL). Similarly, significant ($p \leq 0.05$) reduced activities of serum alanine amino transaminase (ALT) and LDH on continuous probiotic feeding were also indicative of normal liver/kidney functions as they were in normal range for mice. Further, insignificant changes in macrophage chemoattractant protein (MCP-1) in intestinal fluid irrespective of bacterial dose fed along with significant reduction ($p \leq 0.05$) of tumor necrosis factor-α (TNF-α) at much higher dose (10^{13} cfu/animal/d) also confirmed safe response of probotic *L.rhamnosus* against inflammation. To conclude, the results obtained under *in vitro* and *in vivo* studies has established the *Lactobacillus rhamnosus* as safe and non-toxic to weaning mice as well as human epithelial cells and thus may be used as a safe food additive.

1. Introduction

Probiotics are beneficial live microorganisms which are well documented for immense health benefits [1]. Probiotic microbes especially lactic acid bacteria (LAB) have been in human use from last many years in the form of various fermented foods and cultured milk products without any obvious antagonistic effects [2]. They are usually considered safe and beneficial to the host and thus have become common ingredients of modern day functional foods. In fact, no major safety concern with probiotic intake was reported in adults and even in infants [3]. However serious concerns have been raised for the industrial and commercial use of newly identified probiotic strains as certain lactic acid bacteria (LAB) strains were isolated from patients with endocarditis, sepsis, liver abscesses and urinary tract infections [4]. Lactobacilli and Saccharomyces organisms are also reported with bacteraemia and fungemia especially in patients with innate central venous catheters [5]. To address this predicament, World Health Organization have developed guidelines for the evaluation of probiotics in food which includes strain identification, safety evaluation, and efficacy testing under *in vitro* and in animal models [6]. Therefore, safety assessment has become an important criterion and foremost step for probiotic selection prior to their incorporation into the human food chain. Adherence to the intestinal mucosa transiently or permanently is also an essential requirement for probiotics so as to avoid their clearance from the colon and is promoted by the expression of key proteins *Mub* (mucus-binding protein) and *Map-A* (mucus-adhesion promoting protein) present on surface of probiotics [7,8]. On one hand, successful colonisation and metabolic activity in the host intestine depends upon bile salts tolerance mediated by *bsh* (bile salt hydrolase) activity [9] but on the other it is still a debatable issue as it may hinder fat absorption.

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