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DEVELOPMENT OF THERMO-STABLE AND PH-RESPONSIVE MICROENCAPSULATED *LACTOBACILLUS PLANTARUM* LAB12 FOR TARGETED GUT DELIVERY

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ABSTRACT

Lactobacillus plantarum LAB12, a lactic acid bacteria (LAB) strain isolated from local fermented food, possess probiotic characteristics. In spite of their chemopreventive properties, the vulnerability of LAB12 during gastrointestinal transit (pH and enzymatic action) and industrial processing (heat and storage) remains a major concern. This study addressed these issues by immobilising LAB12, by means of microencapsulation, within alginate (Alg)-based polymeric matrix, with incorporation of xanthan gum (XG) and coated with Ch (Alg-XG-Ch), or pea protein isolate (PPi; Alg-PPi). The physicochemical properties of Alg-based microcapsules were characterised by means of Fourier transform infrared (FTIR), X-ray diffraction (XRD) and differential scanning calorimetry (DSC) analysis. Survivability of microencapsulated LAB12 exposed to simulated gastrointestinal fluids (pH 1.8 and pH 6.8), high temperatures and various storage conditions (4/8-week storage at 4 and 25 °C) were assessed. Pelletisation study was conducted to evaluate the survivability of microencapsulated LAB12 subjected to actual heat challenge in industrial processing. The microencapsulated LAB12 was further assessed for their safety through acute and subchronic toxicity studies *in vivo*. The fate and release of LAB12 from Alg-based microcapsules in different rodent gut sections were examined by means of confocal microscopy and qPCR respectively. The chemopreventive properties of microencapsulated LAB12 were validated using an orthotopic mouse model. The Alg-XG-Ch microcapsules diameter (1299-1343 µm) were relatively outsized (> 350 µm), a feature which could adversely affect sensory properties resulting in inappropriate mouth feel and flavour. Alg-PPi microcapsules, on the other hand were presented with a smaller diameter range (157.7-189.5 µm) and could be an ideal microencapsulation system for LAB12. This was based on their excellent tolerance against simulated gastric juice (96.4% survivability, intense heat (80.2% survivability at 100 °C for 30 minutes), storage (>7 log CFU g^{-1} after 8-week storage at 4 and 25 °C), pelletisation (89.4% survivability) and targeted release in simulated intestinal fluid (>9 log CFU g^{-1}). The Alg-PPi LAB12 microcapsules were used for all the susequent *in vivo* studies. For toxicity studies, no treatment (2.5×10^{10} CFU kg^{-1} BW) related adverse effects were observed in serum biochemistry and blood haematology. Histological sections of vital organs which included heart, kidney, lung, spleen, liver and gonads suggests that LAB12 encapsulated in Alg-PPi were non-pathogenic and safe for consumption. As for the *in vivo* release study, the microcapsules were found intact in the stomach and LAB12 were found to be present abundantly (>7 log CFU) only in the intestines. Also, orthotopic mouse model pre-fed with microencapsulated LAB12 significantly ($p < 0.05$) reduced tumour volume (-98.87%) and weight (-89.27%) when compared to control. The chemopreventive effect could be possibly attributed to apoptosis and antiangiogenesis mediated, at least in part, through up-regulation of p53 (+32.50%) and caspase-3 (+92.61%), and down-regulation of COX-2 (-63.96%), VEGF (-65.93%) and PECAM-1 (-62.72%). Altogether, this study strongly implied the possibility of having the LAB12-loaded Alg-PPi microcapsules safely incorporated into various food types and nutraceutical products upon succesful completion of clinical trials.

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CHAPTER ONE
INTRODUCTION

The microbial community of human digestive tract contains 10 trillion (10^{13}) to 100 trillion (10^{14}) microorganisms, a total number that is about 10 times greater than that of somatic and germ cells added together (Kim and Lin, 2007). These microorganisms are populated mainly in the colon, with the majority of them being bacteria. This bacterial population is diverse, containing 300 to 500 different species. The gut microflora plays a beneficial role in influencing host physiology and modulation of normal and immune homeostasis (Sommer and Bäckhed, 2013). Dysbiosis, a condition characterised by imbalanced alteration of the body's microbial community, can be potentially caused by detrimental microorganisms and become the root cause of many diseases (Ahn et al., 2013). Transient dysbiotic enteropathogens such as *Salmonella* spp., *Helicobacter pylori*, *Escherichia coli*, *Campylobacter* spp. and *Listeria* spp. possess sufficient virulent properties that can cause Crohn's disease, ulcerative colitis and colorectal cancer (CRC) (Frank, Zhu, Sartor and Li, 2011). Given the microbiota-health relationship, dysbiosis can still be reversed in favour of a balanced gut microbiota. In fact, there is now increased interest in modulating imbalanced gut microbiota through deliberate ingestion of live beneficial bacteria, more widely known as probiotics.

Probiotics are ‘‘*live microorganisms that, when administered in adequate amounts, confer a health benefit on the host*’’(FAO/WHO, 2002). They are mainly lactic acid bacteria (LAB) that serve as commensal bacteria for maintenance of a healthy intestinal environment as well as regulation of the host's physiological homeostasis and health development. In fact, the health promoting benefits of LAB have been recognised and explored for over a century. To date, lactobacilli and bifidobacteria are the two commonest LAB genuses that have been extensively studied for their therapeutic and beneficial effects (Allen et al., 2013; Vlasova, Kandasamy, Chattha, Rajashekara and Saif, 2016). *Lactobacillus casei*, *L. acidophilus*, *Bifidobacterium bifidum*, *B. lactis* (alternatively known as *B. animalis*), *B. longum*, *B. breve*, and *B. infantis* are some of the LAB commonly incorporated into various food products (Anal and Singh, 2007; Kumar et al., 2015). Consumption of LAB, which has led to significant improvement